1-1-2015

Improvements to Broiler Production through Amino Acid Digestibility and Feed Additives

Cameron Mario Cardenas

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By

Cameron Mario Cardenas

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture
in the Department of Poultry Science

Mississippi State, Mississippi

August 2015

Improvements to broiler production through amino acid digestibility and feed additives

By

Cameron Mario Cardenas

Approved:

_____

Kelley G. S. Wamsley
(Major Professor)

_____

Wei Zhai
(Committee Member)

_____

Elizabeth J. Kim
(Committee Member)

_____

Christopher D. McDaniel
(Graduate Coordinator)

_____

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Cameron Mario Cardenas

Date of Degree: August 14, 2015

Institution: Mississippi State University

Major Field: Agriculture

Major Professor: Dr. Kelley Wamsley

Title of Study: Improvements to broiler production through amino acid digestibility and feed additives

Pages in Study: 102

Candidate for Degree of Master of Science

Improvements to broiler production can be made through the means of nutrition and preventative health care. Formulating diets based on a digestible amino acid (AA) basis decreases feed cost and improves bird performance by providing a more accurate diet based on the bird's specific production or age nutrient requirements. The apparent ileal amino acid digestibility (AIAAD) assay utilized in Experiment 1 demonstrated inconsistent results for age effects on AIAAD of an animal by-product blends for specific AA or age. Increasing pathogenic resistance and consumer's push on the market, has caused integrators to incorporate alternative medications or inclusion strategies to coccidiosis management. Experiment 2 demonstrated diets containing virginiamycin, with an inclusion of salinomycin during d 14-28 only, decreased lesion scores and improved performance of birds receiving an increased dose of live coccidiosis vaccine. In Experiment 3, birds fed diets utilizing an ionophore regimen and additional algal beta-glucan demonstrated improved live performance.

DEDICATION

First, I would like to thank my major professor Dr. Kelley Wamsley. I cannot tell you how much I appreciate your advice and words of wisdom. It has truly helped me grow as a student and person. Thank you again for the incredible opportunity.

Dr. Wei Zhai and Dr. Elizabeth Kim, thank you for being great mentors. Dr. Zhai, your nutrition class was one of my first experiences here at MSU and I truly enjoyed our small class lectures. Dr. Kim, thank you for always being so friendly and always providing words of encouragement at the pellet mill.

I would like to thank Donna Morgan, my lab technician. You have taught me many things over the last two years. I'm not sure if I would have kept my sanity while using the bomb calorimeter or ELISA kit if it were not for you.

A big thank you to my lab mates, Ben Sellers and Rosana Hirai. It has truly been a pleasure working with you. I cannot tell you how much I have learned about valuable team work from our experiences. Also, thank you Melissa Triplett. Thanks for the daily positive attitude and being such a great friend.

Finally, I would like to thank my family. You have helped me become the man I am today. Without you this achievement would not have been possible. Mom and Dad, thanks for always providing words of encouragement and believing in me.

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CHAPTER I

LITERATURE REVIEW

Evolution of the Chicken into America's Favorite Protein

The modern chicken did not always display the characteristics it does today. The more territorial ancestor, the Red Junglefowl (*Gallus gallus*), is believed to have been domesticated over 8,000 years ago in Southeast Asia [1]. Over time, the modern day chicken (*Gallus domesticus*) has become a leader in meat production through the use of natural and artificial genetic selection. Natural selection is a slower process in which biological trait improvement occurs through long cycles of reproduction. By using artificial selection, scientists have been able to accelerate the process by breeding birds that display beneficial traits such as improved feed conversion ratios, increased body size or, muscle accretion [2]. This, along with improvements made in the area of poultry nutrition and environmental management, have allowed poultry to become an affordable, highly concentrated source of quality protein. Therefore, broiler chicken consumption per capita has increased each year since the early 1960's; today, Americans consume approximately 89 pounds per capita of chicken products [3].

Production of Poultry Feed

In the poultry industry, feed and feed manufacture are the most expensive aspects, contributing to close to 70% of the total cost of poultry production. These costs go

towards producing a feed that will meet the bird's nutritional requirements, ensuring that they grow quickly and properly. This means meeting digestible amino acid, energy, mineral, vitamin, and water requirements. As poultry nutritionists, we formulate the diet based on the price, availability, and nutrient profile of the ingredients [4]. Poultry diets use a variety of ingredients, such as cereal grains, fats, plant protein sources, animal by-products, vitamin and mineral supplements, and amino acid supplements [4]. Corn and fat sources, such as poultry grease, are most commonly used as the primary ingredients to meet energy needs [4]. Soybean meal is commonly the primary plant protein source [4]. Animal by-product blends, including meat and bone meals, are often used as animal protein sources that can help meet amino acid requirements of chickens [4]. Other ingredients commonly incorporated include limestone, rock phosphate, and vitamin/micromineral premixes [4].

Poultry diet formulation

To formulate diets, the nutritional requirements of the growing chicken and accurate nutrient profiles of each ingredient must be known. This proves to be difficult, as many of the common feed ingredients can vary in nutrient content and digestibility, depending on manufacturing techniques or crop harvest location [4, 5]. Additionally, nutrient requirements can change with the stage of bird development or age.

Traditionally, poultry diets were formulated to meet crude protein and total amino acid requirements; however, formulating diets on a total amino acid basis can be considered an over-estimation and diets are now formulated on a digestible amino acid basis [6]. Digestible amino acid content is one of the most important factors used to formulate broiler diets, as muscle accretion is the main production goal for broilers.

Diets formulated on a digestible amino acid basis can decrease feed costs, as well as more accurately supply amino acids; thus, avoiding over-formulation and nutrient waste [7]. As previously mentioned, dietary protein can be provided by various ingredients including soybean meal, meat and bone meals, and various animal by-product blends. Due to research in Chapter 2 investigating animal by-product blends and digestibility of amino acids this literature review will provide background information on the importance of amino acids for poultry and the process of creating animal by-product blends.

Essential and Nonessential Amino Acids of Poultry

Amino acids are commonly referred to as the "building blocks" of proteins, DNA, and RNA and can be arranged in many different confirmations to create a variety of biological molecules. Structural proteins can found in the feathers, skin, ligaments, bones, and several organs or muscles [4]. Amino acids can also play a role in metabolic pathways. In times or high stress or trauma, amino acids can be converted to fuel sources [4]. Proper supplementation of amino acids is also vital to ensure broiler growth and muscle accretion. Because the broiler chicken is continuously growing and increasing in muscle mass, it is of utmost importance that diets are formulated to meet the recommended amino acid requirement so that genetic potential is maximized.

Amino acids can either be classified as essential or nonessential. Essential amino acids must be supplied in the diet and cannot be synthesized at all or at a sufficient rate [4]. Essential amino acids for poultry include the following: methionine, lysine, threonine, arginine, histidine, leucine, isoleucine, phenylalanine, tryptophan, and valine. Nonessential amino acids can be created by other amino acids inside the body and not necessarily required in the diet; however, that does not mean they should be completely

disregarded from the diet [4]. These include the remaining amino acids: cysteine, alanine, proline, serine, tyrosine, aspartic acid, and glutamic acid. If there is not a sufficient concentration of nonessential amino acids in the chicken's body, essential amino acids will be converted to meet requirements, which may affect growth/muscle accretion [4].

Methionine and lysine are considered to be the first and second limiting amino acid, respectively, in poultry nutrition [4]. A limiting amino acid may be described as an inadequate amount of an essential amino acid in a diet that decreases protein synthesis to the level at which the amino acid is available [4]. Methionine is very important for several biological functions because it can readily donate its methyl group, and of most importance, can play a vital role in creating sulfur bonds for feather production [4]. The second limiting amino acid, lysine, is considered limiting because the primary ingredient in US broiler diets, corn, is relatively low in digestible lysine [4]. Ultimately, if poultry nutritionists do not formulate diets that meet the amino acid requirements of the growing chicken, producers may notice decreased breast and body weight which ultimately affects the company's total profit. To help alleviate concerns with limiting amino acids, poultry nutritionists can incorporate feed ingredients that have relatively high concentrations of crude protein and amino acids, such as animal by-product blends.

Animal by-product blends

Animal by-products are a common source of digestible amino acids. These blends are created by blending meat and bone meals, fish meals, and/or poultry by-product meals obtained from the rendering facilities. These rendering plants are located close by to poultry processing plants, making it readily available to broiler integrators.

Biologically, many parts of the animal are edible after the proper processing, but these parts are not directly consumed by humans. Parts that are broken down and processed into value-added products include bones, fat, blood, feathers, and organs such as the heart and lungs.

Creation of the by-product meals is achieved through several steps [8]. First, the structural proteins need to be extracted; this can be achieved with solvents such as sodium chloride or solutions high in pH. The condensed protein content is then removed by use of a large centrifuge and screen, followed by the drying and pressing process, to remove excess moisture and fat. Some rendering facilities incorporate additional high cooking temperatures and/or grinding for specific animal by-product blends. High temperatures used during the rendering process help denature proteins and decrease pathogenic bacteria to an extent [8].

Research has shown that cooking temperatures can affect the digestibility of amino acids in the by-product blends. Processing temperatures of 150 °C can significantly reduce the availability of lysine in meat and bone meals [9]. Increased cooking temperatures during the rendering process can cause some amino acids to bind to free sugars via Maillard reactions, thus, decreasing the digestibility [10]. Increased cooking time, with high temperatures, can also decrease the digestibility of amino acids in meat and bone meals as reported by Johns and colleagues [11]. It is important to note that the cooking temperature and time may decrease the digestibility of the limiting amino acids, methionine and lysine. As previously mentioned, these two essential amino acids are commonly referred to as limiting due to the relatively low levels of methionine in soybean meals as well as its use for feather production and relatively low lysine

content found in corn, a major ingredient incorporated into poultry diets to meet energy requirements [4].

As noted, these animal protein sources can vary in composition due to manufacturing techniques and specific animal sources [5, 10, 11]. Therefore, to evaluate the digestibility of common feedstuffs certain biological assays may be conducted, such as the precision fed cecectomized rooster assay (PFR) or the apparent ileal amino acid digestibility assay (AIAAD).

Precision fed cecectomized rooster assay

The precision fed cecectomized rooster assay is considered to be a balance digestibility assay. This type of assay has been used for over 25 years and is considered to be less expensive and all amino acids can be evaluated [13]. Before conducting this assay, the ceca must be surgically removed to avoid overestimations of digestibility caused by cecal microbes; the removal of the ceca can be a large initial investment. Garcia et al. [14] raised concern that this assay might not accurately report the AA digestibility of growing animals, because the rooster assay is conducted on birds that are considered physiologically mature. Also, precision feeding is not considered to be a normal eating behavior because birds do not have ad libitum access to feed [14].

The procedure of the PFR has be reported in many studies [12-14]. To conduct the assay, a rooster's feed must be withdrawn for 24 hours. The bird is then tube fed approximately 30 g of the feed sample via the crop. All excreta and urine voided is then collected over a 48 hour period. Endogenous corrections can be made by fasting roosters for 48 hours and collecting the excreta. Following collection, excreta is frozen, freeze dried, weighed, ground, and then analyzed for the amino acid content. In addition, feed

samples are also analyzed for amino acid content to determine amino acid intake. The amino acids can be standardized with the following equation [12]: Standardized amino acid digestibility % = [(amino acid fed, mg – amino acid excreted, mg + endogenous amino acid excreted, mg) / amino acid fed, mg] × 100. Due to some of the concerns with the PFR assay, the apparent ileal amino acid assay (AIAAD) has increased in popularity.

Apparent Ileal Amino Acid Assay

This bioassay utilizes semi-purified diets with indigestible markers and the test ingredient supplying all amino acid and crude protein requirements [15-17]. Birds are fed common commercial diets until the experimental phase begins. Birds are then moved to battery cages so that they have ad libitum access to semi-purified diets and water. Experimental phases utilize a 24 hour fasting period and then allow for the birds to consume the semi-purified diets for 72 hours prior to the respective day of AIAAD determination [16, 17]. Birds are then euthanized and ileal digesta can be collected either from the total tract or terminal end [16, 17]. Digesta samples can be pooled by replicate cages, homogenized, freeze-dried, ground, and then frozen until processing [15, 17]. Feed and digesta are then analyzed for amino acid and indigestible marker concentrations. The following equation [7] is used to determine AIAAD %: [1 – (marker in diet / marker in ileal digesta) × (amino acid in digesta / amino acid in diet)] × 100. These digestibility coefficients are then used to calculate digestible amino acids and allow for improved formulation of broiler diets.

This assay can be considered more beneficial because it can account for bird age and/or strain effects on amino acid digestibility and utilize a more natural feeding program, as opposed to a physiologically mature rooster or precision feeding [14]. The

assay generally estimates the amino acid digestibility of broilers at 21 days of age and these values are typically used throughout dietary phases. However, more recent research has utilized more than one age to determine possible age effects on digestibility [16, 17]. Age effects on amino acid digestibility will be further discussed in Chapter 2 of this thesis.

Improvements to broiler production may also be made through the use of many different feed additives. In the interest of research in Chapters 3 and 4, feed additives used to help control and prevent coccidiosis in a commercial setting will be discussed in further detail in the following sections.

Feed Additive Impact of Anticoccidials or Growth Promoters on Coccidiosis and Subsequent Broiler Performance

Coccidiosis

Coccidiosis is a gastrointestinal disease caused by a protozoan parasite of the *Eimeria* genus [18]. The disease can infect most wild or domestic animals, but each species of *Emeria* are species specific. Species of concern for the poultry industry include *E. acervulina*, *E. brunette*, *E. maxima*, *E. mitis*, *E. praecox*, *E. tenella*, and *E. necatrix* [19]. However, due to the focus of this thesis and most vital to the poultry industry, only *E. acervulina*, *E. maxima*, and *E. tenella* will be discussed in detail.

This parasitic disease is responsible for economic losses for many different food producing animal industries around the world, primarily decreasing overall production by affecting body weights and feed efficiency. In the commercial broiler industry, in which integrators are trying to maximize muscle accretion and feed efficiency, coccidiosis incidence has escalated due to stocking density and potential resistance to antibiotics and

anticoccidial drugs [20, 21]. Research has estimated the economic losses vary but can range 1-3 billion dollars worldwide per year, primarily due to the measures taken to provide preventative care [22, 23]. Before we can truly understand how to control coccidiosis we must understand the pathogenicity of *Eimeria* parasites.

Infection, diagnosis, and symptoms

Coccidiosis infections are caused by motile protozoan parasites of the *Eimeria* genus. These parasites are prevalent in almost all areas poultry are raised. They can be found in litter, water, feed, and on various surfaces, making it extremely hard to eradicate from the environment [19]. Understanding the life cycle of these parasites is crucial in determining effective prevention and control.

The life cycle was first described by Fantham [24]. At the beginning of the life cycle, oocysts are found in the poultry droppings and litter. Only under the proper conditions of moisture and oxygen do the fertilized oocysts develop four sporocysts that contain two sporozoites each. This stage of development is called sporogony. After this stage, oocysts are ingested by the chicken. The sporocysts membranes are then deteriorated by intestinal secretions; thus, releasing the sporozoites into the intestinal lumen. The sporozoites then penetrate the intestinal cells and develop into schizonts, containing merozoites. This is considered the asexual reproduction or schizogony stage of development. The merozoites are motile and can continue to invade adjacent cells thus continuing to multiply; causing further damage to the gastrointestinal tract. In the final stages of development, merozoites differentiate into male and female and fuse to create fertile oocysts. This is considered to be the gametogony or sexual reproduction phase of development. Fertilized oocysts are then released into the environment via

poultry droppings to restart the *Eimeria* life cycle. This same detailed cycle has also been reported by Johnson [18].

Johnson [18] has made many other significant contributions to the area of coccidiosis research. He first reported that certain species of *Eimeria* can only infect chicken and therefore are host specific [18]. He also notes that there are differences in shapes and sizes of oocysts, which has helped with the discovery of different species of *Eimeria* [18]. It is important to note that each species of *Eimeria* that infect chicken will reside and develop in different parts of the gastrointestinal tract and thus cause different types of lesions and damage to the intestinal lining [18, 20, 25]. The following subsections will discuss the site of infection, diagnosis of species, and symptoms of the three *Eimeria* parasites that are of utmost importance to the broiler industry.

Eimeria acervulina

Eimeria acervulina was first described by Tyzzer in the American Journal of Hygiene [25]. Colonization of the first part of the intestine (duodenum) is a primary characteristic of *E. acervulina*, most noted by the thickening of the duodenal tissue and small white spots or bands in the intestinal mucosa [26]. More severe lesions cause the mucosa lining to begin sloughing and therefore, cause decreased absorption of nutrients. Successful diagnosis is achieved through the appearance of location specific lesions as well as examining intestinal scrapings under a microscope [26]. General symptoms, including depression, inappetance, decreased weight gain, and diarrhea, are comparable to other coccidiosis infections and; therefore, symptoms can only provide speculation until post mortem lesions can be studied [26].

Eimeria maxima

Tyzzer was first to report a new species of *Eimeria* when he isolated *Eimeria maxima* from chickens in 1929 [25]. This species is characterized by the location of infection and post mortem lesions most commonly found in the jejunum [20, 25]. Depending on the magnitude of infection, lesions can consist of inflammation/thickening of the jejunum or more severe hemorrhages of the mucosa [20]. Diagnosis is confirmed by lesion scoring; oocysts viewed under a microscope are often much larger than other *Eimeria* species [26]. Another differentiating lesion can be described as orange or pink viscous content in the jejunum tract [26]. Common to most species of *Eimeria*, clinical signs or symptoms include depression, inappetance, decreased body weight, and decreased feed efficiency [25, 26].

Eimeria tenella

Often considered to be the easiest to diagnose, *Eimeria tenella* was first reported by Ralliet and Lucet in 1891 [25, 27]. It can be easily classified by the location specific lesions and hemorrhage found in the cecal pouches [26]. In extreme cases of infection, *E. tenella* can extend into the lower small intestine and even the duodenal loop. Post-mortem lesions found in the ceca help lead to a positive diagnosis. Cecal walls are often thick and can appear white due to the abundance of oocysts in cecal glands [25]. In addition, upon necropsy, large amounts of blood and necrotic material can be found within the ceca. Symptoms of infection are similar to other coccidiosis infections (inappetance, depression, and decreased body weight) and can lead to a difficult diagnosis by visual symptoms alone; however, characteristic of *E. tenella* infections, large amounts of bright red bloody excreta can be found five days after infection [25, 26].

Control and Prevention of Coccidiosis

Controlling coccidiosis can be achieved through a number of management practices. In the United States, broiler integrators commonly remove caked litter and top dress houses with new fresh shavings. There is also a down period between flocks in order to aerate the house and litter. In some European countries, a complete total clean out is utilized between each grow-out [20]. However, as previously mentioned, *Eimeria* oocysts are virtually everywhere; thus, removal of litter cannot be the sole source of coccidiosis control. Therefore, the use of coccidiosis vaccines, antibiotics and anticoccidials, and newer alternative products to combat coccidiosis have become more important and prevalent in today's poultry industry [28].

Coccidiosis Vaccines

Coccidiosis vaccines have been utilized by the poultry industry since the first commercially available successful vaccine was created by S. A. Edgar in 1952, at Auburn University [29]. This vaccine contained live, sporulated *E. tenella* oocysts and helped to provoke an immune response in chicks. Throughout the years, the coccidiosis vaccine has evolved to include more than one species of *Eimeria*, as well as multiple modes of application [29]. Today, live coccidiosis vaccines are commonly applied to chicks at the hatchery or on the farm as live virulent or attenuated vaccines. Live virulent vaccines often contain varying amounts of wild-type *Eimeria* strains and must be applied evenly, in low doses, to avoid detrimental effects to live performance [30]. Live attenuated vaccines are more preferable due to the attenuation process reducing the reproductive cycle and thus decreasing risk of clinical infections [30-32]. Positive effects of live attenuated vaccines on growth performance and control of clinical coccidiosis have been

reported to be comparable to some anticoccidials [33]. Most vaccines can either be sprayed at the hatchery or introduced via the feed or water [34]. Either mode of application requires ingestion of the infective oocysts provided in the vaccine. Furthermore, today some live coccidiosis vaccines utilize older *Eimeria* oocysts that were isolated before anticoccidials were found to be effective for coccidiosis control [34]. These oocysts are inherently more susceptible to anticoccidials and therefore have been found to be effective at restoring drug sensitivity while still eliciting an immune response [34].

Anticoccidials: Polyether Ionophores

Anticoccidial use has be prevalent in the poultry industry for the past five decades [35]. These feed additives, more specifically ionophores (polyether antibiotics), act as bridges or shuttles to allow ion exchange across cell membranes thus destroying pathogenic cells [35]. These feed additives are commonly utilized by integrators today due to their broad spectrum of protection against many *Eimeria* species. [36]. The slower adaptation to becoming resistant to ionophores vs. antibiotics, stems from the wide variety of ionic pathways, whether it be transport of cations or anions, demonstrated by many different ionophores. In addition, integrators today use a rotation or shuttle program that also helps slow the onset of pathogen resistance [34, 37]. Many different ionophores are available today for commercial use; however, the specific ionophores salinomycin and lasalocid will be discussed in greater detail on account of previous literature describing their benefits of improving live performance and combating coccidiosis while under experimental challenge.

Salinomycin

Salinomycin is a polyether antibiotic that facilitates the transport of sodium and potassium ions across cell membranes of gram-positive bacteria, mycobacteria, some fungi, and more specifically *Eimeria* parasites [37, 38]. Unlike synthetically created drugs, this ionophore is a product of *Streptomyces albus* fermentation [38, 39]. Its efficacy has be widely reported throughout previous literature in providing growth performance and decrease of coccidiosis lesions and oocysts [37, 39, 40].

In early research conducted in 1979, salinomycin was found to be comparable or superior to monensin or lasalocid when fed to *Eimeria* infected broilers in battery cages [39]. This study demonstrated the efficacy of controlling lesions of several *Eimeria* species, including *E. acervulina*, *E. maxima*, *E. tenella*, and a mixed inoculum when compared to infected control birds. Additionally, when compared to infected control birds, salinomycin supplementation demonstrated a 40 to 60 gram improvement in body weight gain [39]. Effects of salinomycin are importantly noted in this battery cage trial; however, use of floor pens where birds are raised on litter and have direct contact with feces and oocysts can provide a more practical experimental environment.

A study conducted at the University of Georgia under McDougald and cohorts utilized this practical design of floor pens [40]. After infection with *Eimeria* parasites, birds fed diets containing salinomycin demonstrated reduced FCR comparable to monensin and lasalocid, but significantly lower than birds fed unmedicated diets [40]. The FCR of birds fed salinomycin corresponded to the increased body weights observed on day 28 and 35 when again compared to the body weights of unmedicated chickens [40]. The study further described decreased lesion scores by noting while comparable to

other anticoccidials, monensin and lasalocid, salinomycin demonstrated the ability to reduce average total lesion scores [40].

Due to concerns with long term use and subsequent increased pathogen resistance as well as advancements in genetics and nutrition it is important to note the efficacy of salinomycin still holds true today. Abdelrahman et al. reported several effects on birds fed salinomycin while under a coccidiosis challenge [37]. On day 42, birds demonstrated a significant increase in body weight when compared to infected unmedicated control birds, as well as a significant reduction in FCR [37]. Furthermore, anticoccidial effects of salinomycin were demonstrated by the significant reduction of oocysts shed in feces on day 28 [37]. In agreement with this research, Ritzi and cohorts also reported salinomycin improved day 21 body weight, FCR during day 15 to 21, and decreased duodenal lesion scores when compared to unmedicated infected control birds [41].

Lasalocid

Lasalocid is considered to be one of the many ionophores used today to combat coccidiosis infections. This ionophore also allows for bidirectional exchange of free ions, such as potassium and sodium, across the cell membranes [42]. Lasalocid is a product of *Streptomyces lasaliensis* fermentation [42].

Lasalocid was first reported to have effects on poultry coccidiosis in 1974 by Mitrovic and Schildknecht [42]. They reported that lasalocid was successfully included into basal diets fed to birds infected with *E. tenella* and reduced the mortality and negative effects on weight gain when compared to birds infected and fed unmedicated diets [42]. These same effects were noted for birds infected with *E. acervulina* and *E. maxima* as well [42]. Mitrovic and Schildknecht continued to compare lasalocid to

several other anticoccidials in 1975 and found that it either performed equally or superior to previously used anticcodials in the industry [43]. In a more recent study, lasalocid inclusion vs. unmedicated diets improved day 42 body weight of broilers and decreased oocyst concentrations found in litter [44]. These results are comparable to research conducted by Mathis and cohorts [45]. They reported that lasalocid fed during day 18 to 50 or 35 to 50 significantly improved body weight of *Eimeria* vaccinated chicks when compared to vaccinated control birds [45]. This report also demonstrated the efficacy of decreasing lesion scores at day 35 caused by *E. acervulina*, *E. maxima,* and *E. tenella* [45].

Alternative Products to Combat Coccidiosis

Due to the rising concern of increased resistance to anticoccidials, the research of alternative medications has increased. The use of live coccidiosis vaccines to restore drug sensitivity has already been mentioned and can prove to be effective [33]; however, consumer concern with zoonotic diseases and residues found in poultry meat has caused many integrators to consider migrating to natural feed additives [46]. These feed additives of interest (probiotics, essential oil blends, and beta-glucans) have been demonstrated to maintain live performance and in some cases even improve live performance when compared to medicated and unmedicated diets under a variety of environmental conditions.

Probiotics

Probiotics are defined as nonpathogenic, direct-fed microbials that can help maintain a balance of normal microflora in the gastrointestinal tract [47]. These products

can contain many different strains of bacteria such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Pediococcus*, and *Bacillus* [41, 48, 49]. Probiotic use in the poultry industry has increased in popularity due to the benefits observed in regards to bird performance, intestinal health, and immunity [41, 48]. This is established through different modes of action including competitive exclusion and stimulation of the immune system [50]. Due to the consumers push for more natural meat products [46], these products can be utilized by integrators progressing towards an antibiotic free management procedure.

In regards to coccidiosis, probiotics have been found to decrease lesion scores in the duodenum, jejunum, and ileum caused by various species of *Eimeria* [41, 48, 51]. The proposed mode of action is that these probiotic bacteria are competing for attachment sites on intestinal epithelial cells. Therefore, the motile and invasive stages of *Eimeria* reproduction may not be as effective in invading cells, reproducing, and creating more oocysts to be cycled into litter. Ritzi and cohorts demonstrated this by observing decreased day 20 to 24 oocysts shedding in litter from birds challenged with *Eimeria spp*. and fed a probiotic diet vs. birds challenged and fed unmedicated diets [41]. These positive effects on the gastrointestinal tract can potentially have an effect on the live performance. The direct decrease in oocysts shed and improved body weight was reported by Lee et al. when birds were infected with *E. acervulina* and fed diets containing a *Pediococcus* based probiotic [48]. Bozkurt and colleagues have reported that a probiotic added to basal diet performed similarly, in regards to day 1-42 body weight gain, to the anticoccidial salinomycin during an *Eimeria spp*. challenge [51].

Essential Oil Blends

Natural plant blends that included essential oil blends could also help control coccidiosis in the poultry industry. These blends can contain varying concentrations of essential oils due to the different sources of herbs or plants used for production [52]. Oregano oil has been used in the poultry industry to promote growth because of its potent chemical nature. This oil contains caravcol and thymol, which have been shown to have antibacterial properties towards *Salmonella* enteritidis and *Campylobacter jejuni* in vitro [53]. However, the antibacterial nature of oregano components does not mean that it will provide live performance benefits. Previous research has shown that dietary oregano essential oil did not improve body weights, but did decrease day 21 to 42 and 1 to 42 FCR [54]. However, Christaki et al. did report anticoccidial benefits and improved performance with supplementation of an essential herb and oil blend during an *E. tenella* challenge [55]. They noted that the blend improved body weight gain and FCR of birds during the period of day 0 to 35 when compared to the infected control group [55]. The blend demonstrated anticoccidial effects by significantly decreasing the oocysts excreted when compared to the infected control group [55]. The reduction of oocyst excretion has also been reported by Bozkurt et al [51], thus providing more evidence that essential herb and oil blends could possibly be utilized as alternatives to anticoccidials.

Beta-Glucans

The use of beta-glucan products in the poultry industry has been growing in popularity, not only due to the relatively low footprint of producing the products, but also due to the wide variety of sources, immunomodulation characteristics, and benefits to live performance of broilers [56]. Beta-glucans are long chain polysaccharides composed of

glucose monomers.[56]. However, there are differences in their degree of branching, solubility, molecular weights, and even polymer charge which provide different immunomodulation effects [56]. Primary sources of beta-glucans used in the poultry industry include barley, wheat, oat, yeast (*Saccharomyces cerevisae*), and, increasing in popularity, algae [56]. While there is little published research on algal (*Euglena gracilis*) derived beta-glucans, we do know that the non-branched 1,3 linear linkages can increase its bioavailability and digestibility [57]. Similar in structure, 1,3 linear structure with long chain 1,6 glucose branches, beta-glucans derived from *S. cerevisae* have been reported to provide benefits to chickens through their immunomodulating characteristics. Studies have shown that these characteristics can include the regulation of key cytokines and magnification of immunoglobulin G in blood serum [58], increased cytotoxic t cells and TCR 1 cells [59], and decreased lesion scores caused by *Eimeria spp.* [60]. Some of these positive immunomodulation observations also lead to improved performance. Zhang and cohorts reported that beneficial growth in terms of FCR and average daily gain were noted along with the increase of cytokines and IgG in serum [58]. However, despite positive immunomodulation effects noted, Chae and colleagues and Cox et al. did not notice any positive or detrimental effects on live performance in terms of body weight or body weight gain [59, 60].

Antibiotic Growth Promoters

During the 1940's, the poultry industry experienced a rapid expansion due to the advancements made in genetics, housing environment, nutrition, and importantly the dawn of antibiotic use for growth promotion. As the world's population grew, poultry consumption increased and became an affordable quality source of animal protein. With

the aforementioned advancements in the poultry industry, an increased stocking density observed in commercial broiler houses helped supply the demand for chicken products. This has been made possible due to the use of antibiotics as growth promoters [46]. Today, we know that the modern poultry industry utilizes subtheraputic levels of antibiotics to improve growth and maintain a healthy environment for chickens reared in these intensive conditions. But where did our modern advancements in medication originate?

In 1946, Moore and colleagues reported the first study that demonstrated the effects of antibiotics as growth promoters for chickens. In their study, they found that streptomycin and sulfasuxidine when included to the basal diet alone or in combination successfully increased growth of chicks during the four week study period [61]. Despite the early concerns of antimicrobial resistance, the United States Food and Drug Administration approved the use of antibiotics in production animal feed without prescription in 1951 [28]. Early studies revealed that it was not necessarily the antibiotic absorption by the chicken affecting weight gains and feed efficiency, but the antibiotics effects on the intestinal microbiota [62].

Antibiotic growth promoters serve as a means to control the balance of normal microbiota in the gut [62]. The normal predominately gram-positive bacteria found in the intestinal tract play both positive and negative roles in nutrition, gut morphology, and overall health of the chicken. Primarily, the largest positive role antibiotics provide is the prevention of pathogenic bacteria colonizing the gut through competitive exclusion. This can be achieved by competing attachment sites on the intestinal cells [62].

The effects of antibiotic use for growth promotion has been extensively reported for several decades. However, these observed benefits of live performance and overall health of broiler chickens have not come without consequences. Today, many consumers are worried about antibiotic residue in meat products as well as possible transfer of antimicrobial resistance from animal to human pathogenic bacteria [21, 46]. This has led to the increase of rotation and shuttle programs utilized in the industry today. These programs help decrease the risk of antimicrobial resistance developing by regularly switching between control practices and products [34, 35].

Bacitracin Methylene Disalicylate and Virginiamycin

Bacitracin methylene disalicylate, or commonly referred to as BMD, and Virginiamycin are two popular antibiotics used to combat enteric disease and improve broiler performance. Bacitracin methylene disalicylate, a gram-positive active antibiotic, will also help control lactic acid-producing bacteria. By controlling the levels of lactic acid bacteria more heterogeneous microflora may be able to help control the population of other pathogenic bacteria [64]. This branched decapeptide also disrupts cell walls, cell membranes, and protein synthesis [65]. Research has shown that this narrower spectrum of antibiotic can play a critical role in reducing necrotic enteritis caused by *Clostridium perfringens* [66]. This can be demonstrated by a study that reported significantly lower *C. perfringens* cfu/g in the litter when compared to diets containing virginiamycin [64]. This is important because during a coccidial infection, *C. perfringens* can proliferate in the gut to pathogenic levels and can cause necrotic enteritis, a disease that can cause additional lesions and decrease nutrient utilization [67]. Today, it is common for feeds to

be supplemented with BMD and an anticoccidial to help control coccidiosis and the potential threat of necrotic enteritis.

It has be reported that BMD and narasin, an anticoccidial, when fed alone or in combination can significantly decrease mortality and lesion scores in broilers infected with a necrotic enteritis challenge [68]. Brennan and colleagues also reported that birds fed the BMD and narasin combination or the BMD treatment alone demonstrated increased average daily weight gain and an additional improvement of decreased FCR during day 0 to 21 [68]. They concluded that the reduction of mortality and lesion scores meant BMD's efficacy was not only as an antibiotic growth promoter but it also reduced the clinical disease necrotic enteritis [68].

Furthermore, Miles and cohorts have demonstrated many effects of BMD on intestinal morphology. They reported that supplementing BMD during the study demonstrated decreased muscularis mucosae in the ileum and comparable total villus area vs. unmedicated diets but significantly larger total villus area when compared to virginiamycin diets [65]. This is important because BMD is thought to have an energy sparing effect on tissue maintenance and thinner mucosae is often a descriptive sign of this reduced maintenance requirement [65]. The comparable total villus area suggests that while BMD did not improve absorptive area compared to the control, it did not cause any detrimental effects. Additionally, during this study d 49 BW was also significantly higher when birds were fed diets containing BMD when compared to the unmedicated diets [65].

Virginiamycin, is also considered to be effective against gram-positive and lactic-acid producing bacteria. However, it is thought to have a broader spectrum of activity

than BMD [63]. This antibiotic is a fermentation product of *Streptomyces virginiae* and is considered to be a 2-component streptogramin [64, 65]. The main mode of action for this antibiotic is blocking the synthesis of proteins by binding on two different loci on bacterial ribosomes [65]. Comparable to BMD, this drug can also help decrease negative effects due to necrotic enteritis.

George and colleagues inoculated male broiler chickens with *C. perfringens* at 14 days of age [69]. They subsequently tested five levels of virginiamycin fed in the basal diets. Each level of virginiamycin inclusion decreased the lesion scores and mortality % in infected chickens when compared to the unmedicated infected control group [69]. Additionally, this study also noted improvements to day 35 average body weight and feed efficiency at all levels of virginiamycin when again compared to the unmedicated infected group [69]. To supply additional support to their study, George and cohorts provided two replications for the complete study. In terms of reduced lesions scores and mortality %, as well as improved day 35 body weight and feed efficiency, the two replicated studies demonstrated the same virginiamycin effect when compared to the unmedicated infected group [69].

The growth performance benefits demonstrated in the previous study are consistent with many other reports throughout the literature. March and cohorts found that birds fed diets containing virginiamycin demonstrated a 10.3 and 5% increase in day 26 and 53 bodyweight, respectively [70]. They also reported that FCR were improved by 9.5 and 3.9%, respectively, for day 26 and 53 [70]. This research is consistent with more recent research that compared the effects of BMD and virginiamycin [65]. The efficacy of BMD have been previously mentioned for this study; however, in the same study the

effects of virginiamycin on growth performance (BW and FCR) were numerically better than BMD and significantly higher than the basal diet control [65]. For instance, at day 49 body weights of birds that were fed virginiamycin were comparable to BMD fed birds; however, both of these treatments had increased growth when compared to birds fed the control basal diet without any antibiotic addition [65]. Additionally, birds that were fed virginiamycin demonstrated similar feed conversions to the treatment containing BMD, but significant improvements when compared to the basal diet [65].

In summary, improvements to broiler nutrition can be made in a variety of ways and can have a large impact on the total cost to produce poultry. The focus of this thesis is to determine the need for age specific digestibility coefficients using animal byproduct blends, as well as determine some alternative feed additive controls for improving and/or maintaining broiler performance. By utilizing age specific amino acid digestibility values we can formulate diets that more accurately supply the correct amounts of digestible amino acids for a growing broiler. This may ultimately decrease feed costs by avoiding over-formulation and consequently lower nutrient waste. Additional improvements to broiler production can be achieved through the use of various feed additives. Feed additives used to improve performance and control diseases (e.g. coccidiosis) have been prevalent in the poultry industry for many years. Countless hours of research have been dedicated to determining the correct inclusion strategies to effectively combat the disease and still maintain or improve broiler performance. However, due to the increasing consumer concern surrounding possible increased pathogenic resistance to traditional controls, the broiler industry has directed its focus to the testing and utilization of new alternative products. Research conducted in chapter 2 focuses on improvements to

broiler production through age specific amino acid digestibility coefficients for animal by-product blends; while research reported in chapters 3 and 4 investigate new/alternative methods of disease control.

References

1. Fumihito, A., T. Miyake, T., S. I. Sumi, M. Takada, O. Susumu, and N. Kondo. 1994. One subspecies of the red junglefowl (Gallus gallus gallus) suffices as the matriarchic ancestor of all domestic breeds. Proceedings of the National Academy of Sciences.

2. Siegel, P. B., and E. A. Dunnington. 1997. Genetic selection strategies-population genetics. Poult. Sci. 76:1062-1065.

3. Per capita consumption of poultry and livestock, 1965 to estimated 2015, in pounds. National Chicken council. Accessed Jan. 2014. http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/.

4. National Research Council. *Nutrient Requirements of Poultry: Ninth Revised Edition*, 1994. Washington, DC: The National Academies Press, 1994.

5. Parsons, C. M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poult. Sci. 77:1016-1019.

6. Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Anim. Sci. 81.85-97.

7. Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. 60:423-437.

8. Animal By-Product Processing & Utilization. 1999. Ockerman, H. W., and C. L. Hansen. CRC Press. Boca Raton, FL.

9. Batterham, E. S., R. F. Lowe, R. E. Darnell, and E. J. Major. 1986. Availability of lysine in meat meal, meat and bone meal and blood meal was determined by the slope-ratio assay with growing pigs, rats and chicks, and by chemical techniques. Br. J. Nutr. 55:427-440.

10. Parsons, C. M., K. Hashimoto, K. J. Wedekind, Y. Han, and D. H. Baker. 1992. Effect of overprocessing on availability of amino acids and energy in soybean meal. Poult. Sci. 71:133-140.

11. Johns, D. C., C. K. Low, J. R. Sedcole, M. P. Gurnsey, and K. A. C. James. 1987. Comparison of several in vivo digestibility procedures to determine lysine digestibility in poultry diets containing heat treated meat and bone meals. Br. Poult. Sci. 28:397-406.

12. Kim, E. J., P. L. Utterback, T. J. Applegate, and C. M. Parsons. 2011. Comparison of amino acid digestibility of feedstuffs determined with precision-fed cecectomized rooster assay and the standardized ileal amino acid digestibility assay. Poult. Sci. 90:2511-2519.

13. Parsons, C. M., L. M. potter, and J. R. D. Brown. 1982. Effects of dietary protein and intestinal microflora on excretion of amino acids in poultry. Poult. Sci. 61:939-946.

14. Garcia, A. R., A. B. Batal, and N. M. Dale. 2007. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci. 86:94-101.

15. Kim, E. J., P. L. Utterback, and C. M. Parsons. 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91:1350-1355.

16. Huang, K. H., V. ravindran, X. Li, and W. L Bryden. 2005. Influence of age on apparent ileal amino acid digestiblity

17. Kim, E. J., and A. Corzo. 2012. Interactive effects of age, sex, and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. Poult Sci. 91:908-917.

18. Johnson, W. T. 1923. Avian Coccidiosis. Poult. Sci. 2:146-163.

19. Shirley, M. W, A. L. Smith, and d. P Blake. Challenges in the successful control of avian coccidia. Vaccine. 25:5540-5547.

20. Tewari, A. K., and B. R. Maharana. 2011. Control of poultry coccidiosis: changing trends. 35:10-17.

21. Castanon, J. I R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466-2471.

22. Williams, R. 1999. A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int. J. Parasitol. 29:1209-1229.

23. Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis recent advancements in control measures and vaccine development. Expert Rev. Vaccines 5:143-163.

24. Fantham, H. B. 1910. The morphology and life-history of *Eimeria* (coccidium) *avium*: A sporozoon causing a fatal disease among young grouse. Proc. Soc. Lond. 3:672-691.

25. Tyzzer, E. E. 1929. Coccidosis in gallinaceous birds. Am. J. Hyg. 10:269-383.

26. The Merck Veterinary Manual. 2010. Coccidiosis. Pages 2395-2401. 10th ed. Courier Kendallville, Inc. Kendallville, IN.

27. Chapman, H. D. 2014. Milestones in avian coccidiosis research: A review. Poult. Sci. 93:501-511.

28. Jones, F. T., and S. C. Ricke. Observations on the history of the development of antimicrobials and their use in poultry feeds. 2003. Poult. Sci. 82:613-617.

29. Williams, R. B. 2002. Fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Diseases 46:775-802.

30. Shirley, M. W., A. L. Smith, and F. M. Tomley. 2005. The biology of avian *Eimeria* with an emphasis on their control by vaccination. Adv. Parasitol. 60:285-330.

31. Jeffers, T. K. 1975. Attenuation of *Eimeria* tenella through selection of precociousness. J. Parasitol. 61:1083-1090.

32. McDonald, V., M. Shirley, and M. Bellatti. 1984. *Eimeria* maxima: characteristics of attenuated lines obtained by selection for precocious development in the chicken. Exp. Parasitol. 61:192-200.

33. Williams, R. B., W. W. Carlyle, D. R. Brown, and I. A. Brown. 1999. The efficacy and economic benefits of Paracox, a live attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. Int. J. Parasitol. 29:341-355.

34. Chapman, H. D., and T. K. Jeffers. 2014. Vaccination of chickens against coccidiosis ameliorates drug resistance in commercial poultry production. Int. J. Parasitol. Drugs Resis. 4:214-217

35. Chapman, H. D., T. K. Jeffers, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788-1801.

36. Mcdougald, L. R. 1982. Chemotherapy of coccidiosis. Pages 373-427 in The Biology of Coccidia. P. L. Long, ed. University Park Press, Baltimore, MD.

37. Abdelrahman, M. Mohnl, K. Teichmann, B. Doupovec, G. Schatzmayr, B. Lumpkins, and G. Mathis. 2014. Comparative evaluation of probiotic and salinomycin effects on performance and coccidiosis control in broiler chickens. Poult. Sci. 93:3002-3008.

38. Kinashi, H., N. Otake, H. Yonchara, S. Sato, and Y. Saito. 1973. The structure of salinomycin, a new member of the polyether antibiotics. Tetrahedron Letters 49:4955-4958.

39. Migaki, T. T., L. R. Chappel, and W. E. Babcock. 1979. Anticoccidial Efficacy of a new polyether antibiotic, salinomycin, in comparison to monensin and lasalocid in battery trials. Poult. Sci. 58:1192-1196.

40. McDougald, L. R., and K. Keshavarz. 1981. Anticoccidial efficacy of salinomycin (AHR-3096C) and compatibility with roxarsone in floor-pen experiments with broilers. Poult. Sci. 60:2416-2422.

41. Ritzi, M. M., W. Abdelrahman, M. Mohnl, and R. A. Dalloul. 2014. Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. Poult. Sci. 93:2772-2778.

42. Mitrovic, M., and E. G. Schildknecht. 1974. Anticoccidial activity of lasalocid (X-537A) in chicks. Poult. Sci. 53:1448-1455.

43. Mitrovic, M., E. G. Schildknecht, and W. L. Marusich. 1975. Comparative anticoccidial activity and compatibility of lasalocid in broiler chickens. Poult. Sci. 54: 757-761.

44. Stanley, V. G., C. Gray, M. Daley, W. F. Krueger, and A. E. Sefton. 2004. An alternative to antibiotic-based drugs in feed for enhancing performance of broilers grown on *Eimeria* spp. infected litter. Poult. Sci. 83: 39-44.

45. Mathis, G., J. Schaeffer, K. Cookson, J. Dickson, M. Lavorgna, and D. Waldrip. 2014. Effect of lasalocid or salinomycin administration on performance and immunity following coccidian vaccination of commercial broilers. J. Appl. Poult. Res. 23:577-585.

46. Donoghue, D. J. 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? Poult. Sci. 82:618-621.

47. Patterson, J. A., and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

48. Lee, S. H., H. S. Lillehoj, R. A. Dalloul, D. W. Park, Y. H. Hong, and J. J. Lin. 2007. Influence of Pediococcus-based probiotic on coccidiosis in broiler chickens. Poult. Sci. 86:63-66.

49. Lei, X. J., Y. J. Ru, and H. F. Zhang. 2014. Effect of Bacillus amyloliquefaciens-based direct fed microbials and antibiotic on performance, nutrient digestibility, cecal microflors, and intestinal morphology in broiler chickens. J. Appl. Poult. Res. 23:486-493.

50. Ohimain, E. I., and R. T. S. ofongo. 2012. The effect of probiotic and prebiotic feed supplementation on chicken health and gut microflora: A review. Int. J. Anim. Vet. Adv. 4:135-143.

51. Bozkurt, M., N. Aysul, K. Küçükyilmaz ,, S. Aypak, G. Ege, A. U. Çatli, H. Aksit, F. Çöven, K. Seyrek, and M. Çınar. 2014. Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. Poult. Sci. 93:389-399.

52. Lee, K. W., H. Everts, and A. C. Beynen. 2004. Essential oils in broiler nutrition. Int. J. Poult. Sci. 3:738-752.

53. Johny, A. K., M. J. Darre, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayanan. 2010. Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken cecal contents in vitro. J. Appl. Poult. Res. 19:237-244.

54. Alp, M., M. Midilli, N. Kocabagh, H. Yilmaz, N. Turan, A. Gargh, and N. Acar. 2012. The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. J. Appl. Poult. Res. 21:630-636.

55. Christaki, E., P. Florou-Paneri, I. Giannenas, M. Papazahariadou, N. A. Botsoglou, and A. B. Spais. 2004. Effect of mixture of herbal extracts on broiler chickens infected with Eimeria tenella. Anim. Res. 53:137-144.

56. Volman, J. J., J. D. Ramakers, and J. Plat 2008. Dietary modulation of immune function by beta glucans. Physiol. Behav. 94:276-284

57. Levine, R. B. 2014. Algal immunomodulator may improve broiler health. Feedstuffs. June 24, 2014.

58. Zhang, B., Y. Guo, and Z. Wang. 2008. The modulating effect of beta-1,3/1,6-glucan supplementation in the diet on performance and immunological responses of broiler chickens. Asian-Aust. J. Anim. Sci. 21:237-244.

59. Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, T. W. Hahn. 2006. Effects of the supplementation of beta glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291-298.

60. Cox, C. M., L. H. Sumners, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. Immune responses to dietary beta-glucan in broiler chicks during an Eimeria challenge. Poult. Sci. 89:2597-2607.

61. Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, C. A. Elvehjem, and E. B. Hart. 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165:437-441.

62. Dibner, J. J., and j. D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84: 634-643.

63. Ravindran, V., E. T. Kornegay, and K. E. Webb, Jr. 1984. Effects of fiber and virginiamycin on nutrient absorption, nutrient retention and rate of passage in growing swine. J. Anim. Sci. 59:400-408.

64. LaVorgna, M., J. L. Schaeffer, D. Bade, J. Dickson, K. Cookson, and S. W. Davis. 2013. Performance of broiler fed a broader spectrum antibiotic (virginiamycin) or narrower spectrum antibiotic (bacitracin methylene disalicylate) over 3 consecutive grow-out cycles. J. Appl. Poult. Res. 22:574-582.

65. Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476-485.

66. Phillips, I. 1999. The use of bacitracin as a growth promoter in animals produces no risk to human health. J. Antimicrob. Chemother. 44:752-728.

67. Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159-180.

68. Brennan, J., J. Skinner, D. A. Barnum, and J. Wilson. 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poult. Sci. 82: 360-363.

69. George, B. A., C. L. Quarles, and D. J. Fagerberg. 1982. Virginiamycin effects on controlling necrotic enteritis infection in chickens. Poult. Sci. 61:447-450.

70. March, B. E., R. Soong, and C. MacMillan. 1978 Growth rate, feed conversion, and dietary metabolizable energy in response to virginiamycin supplementation of different diets. Poult. Sci. 57:1346-1350.

CHAPTER II

APPARENT ILEAL AMINO ACID DIGESTIBLITY OF VARIOUS ANIMAL BY-PRODUCT BLENDS IN BROILERS 21 AND 42 DAY OF AGE

Summary

Phase feeding and formulating diets on a digestible amino acid basis is a common practice in the poultry industry to allow for more accurate and cost effective formulations that meet the bird's requirements for a specific stage of growth. Because we have become so specific with our formulations, perhaps the next step is to investigate the effects of age on apparent ileal amino acid digestibility (**AIAAD**), to determine if we could become more precise by formulating diets for a particular phase based on the digestibility of the ingredients according to the bird's age. The objective of this study was to determine broiler age effects (d 21 and 42) on AIAAD using eight commercially available animal by product blends (**ABB**). Birds were obtained from the same hatch and ABB were obtained from the manufacturer and analyzed for total AA content and CP. Semi-purified diets were formulated for each of the ABB to contain 20% CP, with all AA provided by each of the ABB. Diets were balanced for all other nutrients with the exception of energy and CP, with cornstarch and dextrose (2:1) completing the diets. Titanium dioxide was added as an indigestible marker at 0.3%. Birds were fed a common diet when not housed in the experimental setting. Experimental diets were then fed for a 72-h period at d 18 to 21 and 39 to 42 after an overnight fast to allow for age

comparisons. Several ABB demonstrated significance for increased AIAAD at d 21 of age as compared to d 42. However, results were not equally consistent to a specific AA (essential or nonessential) or age. Evaluation of the commercially available ABB demonstrated that age may affect the AIAAD and age should be considered for future investigations.

Keywords: AIAAD, broilers, age, animal by-product blends

Description of Problem

Feed costs represent the majority of the cost for poultry production; therefore, nutritionists are always searching for cost sparing methods for formulation. One way that feed costs have been reduced is by formulation diets on a digestible amino acid (**AA**) basis [1]. Along with reducing feed costs, this allows for a more accurate protein and AA content, improved growth performance, as well as decreased nitrogen excretion into the environment [1-4]. Formulating on a digestible AA basis has become easier due to advancements in technology providing rapid analysis of digestible AA. Although, it should be noted that most digestible AA values are based on d 21 AIAAD or cecectomized roosters values and previous research has shown that age can affect the apparent ileal amino acid digestibility of ABB at different ages [5, 6]. Therefore, it is possible that these age differences can affect the phase feeding diet formulations and may allow for cheaper diets, as well as improved production by supplying a more nutrient precise diet.

Another common practice in the poultry industry is for nutritionists to utilized ingredients such as animal by-product blends (**ABB**), due to their cost and mineral content. In addition, ABB contain a variety of AA at different concentrations at a

relatively high CP (>40%) and digestible AA content, making them ideal for determining AIAAD age effects for broiler chickens. Consequently, the objective of this study was to determine the effects of age (d 21 or 42) on AIAAD of eight different commercially available ABB [7].

Materials and Methods

Formulation of Semi-Purified Diets

Semi-purified diet composition, total AA concentrations in the diet, and AA concentrations of tested ABB are reported in Tables 2.1-2.3, respectively. Experimental diets were formulated to contain approximately 20% CP, with the ABB being the sole source of AA in the diets. Crude protein of the ABB was determined prior to the start of the trial by multiplying N content by 6.25. Titanium dioxide was included in the diet as an indigestible marker at 0.5%. All other nutrients, with the exception of energy and protein, were balanced to meet the minimum NRC [8] requirements at 21 and 42 d of age. Cornstarch and dextrose were utilized to fill the remainder of the diet. It should be noted that birds utilized to test the same ABB were fed the same semi-purified diet during both phases to allow for comparisons between the two ages (d 21 vs. 42).

Bird Husbandry

All animal use was approved by the Mississippi State University and USDA-ARS animal care and use committees. Ross × Ross 708 [9] broiler chicks were obtained from the same hatch at a commercial hatchery [10]. At the hatchery, chicks were vaccinated for Merck's disease and Newcastle disease. Upon arrival, chicks were feather-sexed and male chicks were placed in common floor pens until respective experimental phases,

when birds were moved to battery cages [11] for a 72 hour experimental period. When not under experimentation, birds were fed standard broiler diets formulated to meet or exceed NRC [8] nutrient recommendations and offered ad libitum. Birds had ad libitum access to feed and water throughout the entire study.

AIAAD ASSAY

Digestibility assays were conducted to determine the AIAAD of 8 commercially available ABB at two ages, d 21 and d 42. The first experimental period determined AIAAD of broilers at 21 d of age, while the second period determined the AIAAD of broilers at 42 d of age. The first experimental period utilized 512 broiler chicks and the second utilized 320 broilers. All birds remained in floor pens until d 14 and d 35. On d 14 and d 35 birds were randomly allocated to 64 battery cages [11] and cage weights were equalized. During the first experimental period, 8 birds per cage comprised one replicate group and for the second experimental period, 5 birds were placed per cage. Each of the semi-purified diets mixed with a respective ABB were fed to 8 replicate cages. Battery cages were equipped with 1 feeding trough and 1 nipple drinker and housed in a solid climate controlled room. Temperature started at 84°F and decreased in accordance to Ross guidelines [9] until the temperature of 62°F was reached. A period of overnight fasting occurred on d 17 and 38 of age, then birds were fed respective semi-purified diets ad libitum from d 18 to 21 and d 39 to 42. On d 21 and 42, birds and remaining feed per cage were weighed. Birds then were killed via CO_2 inhalation and ileal digesta samples were collected (pooled by cage). These procedures have been adapted from a previous study conducted by Kim and Corzo [5].

The ileum was determined as the portion of the small intestine starting at the Meckel's diverticulum to the ileo-cecal junction. Ileal digesta from birds within a replicate cage was pooled and frozen at -20°C until processed. Digesta samples were then freeze dried and ground using a mortar and pestle and screened through a 1mm screen to create a homogenous mixture.

Chemical Analyses and Calculations

Ileal digesta samples, semi-purified diets (containing ABB), and ABB products were analyzed for AA [12] and titanium dioxide [13] (digesta and semi-purified diets only) content at a commercial laboratory [14]. Apparent ileal amino acid digestibility was determined using the following equation adapted from Lemme et al. [4]: AID, % = [1 – (titanium dioxide in diet / titanium dioxide in ileal digesta) × (AA in digesta / AA in diet)] × 100.

Statistical Analysis

This study utilized a randomized complete block design. The experimental unit was considered to be one cage, with cage location being the blocking factor. Each ABB was analyzed separately using the PROC GLIMMIX procedure to determine age effects on AIAAD [15]. Alpha was then set at $P \leq 0.05$.

Results and Discussion

Age Effects on Essential AA AIAAD

When formulating diets, in a phase feeding program, it is imperative to supply at least the minimum amount of recommended essential AA to meet the genetic potential of the modern broiler. Over-formulation of diets can increase costs and nutrient waste,

while under formulation may decrease growth performance and subsequent meat yields [16]. Methionine and lysine are considered to be the first two limiting AA in poultry diets and therefore should be taken into consideration when formulating diets based on a digestible AA basis, especially during phase feeding programs.

In the current study, the following essential AA demonstrated significantly increased d 21 AIAAD for ABB1 and ABB7, as compared to birds 42 d of age ($P<0.05$; Table 2.4 and 2.10, respectively): Met, Lys, Arg, Thr, Phe, Trp, Val, Ile, Leu, and His. Most notably, Met and Lys demonstrated an approximate 7 and 4%, respectively increase in d 21 vs. d 42 AIAAD for ABB1; as well as an approximate 2 and 4 %, respective increase in d 21 vs. d 42 AIAAD for ABB7. This may be of concern for poultry nutritionist due to most diet formulations meeting Met and Lys requirements with crystalline AA supplementation that can increase feed costs. Additionally, the essential AA Arg demonstrated a 5% significant increase in d 21 AIAAD for ABB5 when compared to birds 42 d of age ($P=0.0116$; Table 2.8).

Opposing results were obtained for four of the tested ABB, whereas d 42 AIAAD yielded a significant increase of approximately 3, 5and 4% for His when compared to birds 21 d of age for ABB2 ($P=0.038$; Table 2.5), ABB5 ($P=0.0007$; Table 2.8), and ABB 6 ($P=0.0056$; Table 2.9), respectively. Additionally, Thr, Trp, Val, Ile AIAAD for ABB8 were affected by age in which birds 42 d of age demonstrated significant increases of approximately 3, 8, 7, and 3%, respectively, ($P<0.05$; Table 2.10) when compared to d 21.

Data obtained for ABB8 are consistent with research that found increased d 42 AIAAD for the AA Thr, Trp, Val, and Ile of an ABB [5]. Further providing evidence for

these specific results, AIAAD of Thr, Val, and Ile in a meat and bone meal (**MBM**) increased in birds 28 and 42 d of age when compared to d 14 [6]. A possible explanation for the observed increase in AIAAD of older birds could be attributed to decreased passage rate and increased length of intestinal tract, allowing for longer nutrient digestion [17]. Heat damage to proteins during the rendering of ABB could also decrease the digestibility of AA in younger birds [18].

However, this does not explain the differences observed in increased d 21 vs d 42 AIAAD of ABB1, ABB5, and ABB7. To understand the differences in age effects on AIAAD for the tested ABB, the relationship between intestinal morphology and the time period of semi-purified diets should be considered. To determine the AIAAD at d 21, semi-purified diets were fed during d 18-21. In relation to this time period, the activity of trypsin and chymotrypsin has been reported to reach maximum at d 11 post-hatch [19] and in addition, nitrogen digestion has been found to increase from d of hatch to 90% at d 14 [20]. It has also been reported that relative growth of the small intestine per day is highest at d 3-8 and subsequently decreases in rate [21]. Thus, by the time semi-purified diets were fed from d 18-21 the maturity of the intestinal tract and expression of proteases may have been adequate for demonstration of increased d 21 AIAAD vs. d 42 for a specific blend and AA.

Age Effects on Nonessential AA AIAAD

Nonessential AA are classified as AA that can be rapidly synthesized by the chicken [8]. They primarily provide nonspecific nitrogen to the body; but more importantly, if they are not provided in adequate amounts, essential AA may be used to synthesize them [8]. The current study found significant increases in AIAAD of several

nonessential AA for several of the tested ABB. Specifically, the AIAAD of Ser, Glu, Pro, Ala, and Tyr demonstrated increased for birds 21 d of age vs. 42 for ABB1 and ABB7. ($P<0.05$; Table 2.4 and 2.10, respectively). Improved 21 d AIAAD for Cys was also found for birds fed semi-purified diets supplemented with ABB7 ($P=0.0489$; Table 2.10). Proline and Ala AIAAD also improved for birds 21 d of age vs. 42 for both ABB3 and ABB4 ($P<0.05$; Table 2.6 and 2.7, respectively). Also, d 21 AIAAD for Pro was enhanced for birds fed semi-purified diets containing ABB5 and ABB6 ($P<0.05$; Table 2.8 and 2.9, respectively).

Research conducted by Kim and cohorts [5] does not agree with the observed differences, as d 21 AIAAD decreased for Ser, Glu, Cys, and Tyr when compared to birds 42 d old. Additionally, Ser demonstrated decreased d 14 AIAAD when compared to d 28 and d 42 [6]. Most notably in the current research, the birds d 21 of age demonstrated a consistent improvement for AIAAD of Pro (Tables 2.4, 2.6, 2.7, and 2.10). Although Pro can be synthesized by the chicken at a rapid rate, the lack of supplementation into the diet can cause decreased body weight gain (**BWG**) and FCR [22]. Poultry nutritionists may not formulate diets used in phase feeding based on digestible Pro, but its supplementation into the diet cannot be ignored completely.

Opposing results were obtained for one of the tested ABB, when in fact birds 42 d of age demonstrated a significantly increased AIAAD of approximately 13, 3, 4, 2, and 4% for Cys, Asp, Ser, Glu, and Tyr, respectively, when compared to d 21 ($P<0.05$; Table 2.11). These specific age effects on AIAAD of Cys, Asp, Ser, Glu and Tyr for ABB8 have also be reported by Kim and cohorts [5] as previously mentioned above; however, for this specific ABB and d of age the data now corresponds with their results. Of most

importance, Cys demonstrated an approximate 14% increase in d 42 AIAAD when compared to d 21. Although there is a sparing effect of Cys on Met, previous research has found that increased supplementation of Cys cannot completely counterbalance a Met deficiency [23].

Overview

Obtained AIAAD of ABB for the current study were inconsistent for specific AA and age. The tested ABB were proprietary, hence it is unclear if differing animal protein sources or concentrations affected the current research results. An additional factor could be the processing temperature damaging protein found in the tested ABB. [18]. Additionally, nutritionists that aim to formulate diets based on a digestible Lys or Met basis may find the increased AIAAD of Lys and Met for birds 21 d of age vs. 42 noteworthy. On d 21, both ABB1 and ABB7 demonstrated a 4% increase in AIAAD of Lys while AIAAD of Met was increased by 7 and 2%, respectively. This may allow for nutritionists to substitute inclusions of more expensive ingredients used to meet digestible Lys and Met requirements for cheaper ingredients while still formulating on a digestible AA basis. It should be noted again, these values are for a specific ingredient and even demonstrated inconsistencies in age and specific AA for the tested ABB. Therefore, more research is warranted in the area of age effects on AIAAD for diet formulations created for different phases of age and growth.

Conclusions and Applications

1. Data from this study suggests that bird age can affect the AIAAD of several commercially available ABB at d 21 and d 42; however, results were not consistent for AA, age or tested ABB.
2. Further investigation of AIAAD values of various feed ingredients for birds at different ages are needed in order for poultry nutritionists to make a more informed decision as to whether or not AIAAD at a particular age should be considered for diet formulation.

Table 2.1 Composition of the semi-purified experimental diets (as-fed basis)[1]

Ingredient (%)	Semi-purified Diet							
	1	2	3	4	5	6	7	8
Cornstarch	18.314	17.469	17.135	16.859	15.365	15.889	14.402	19.066
Dextrose	36.629	34.937	34.271	33.737	30.729	31.777	28.804	38.131
ABB[2]	32.557	35.094	36.344	37.044	41.806	39.984	44.444	30.303
Inert filler[3]	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Poultry Oil	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Vitamin/Mineral Premix	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Sodium Bicarbonate	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Potassium Chloride	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
Magnesium Oxide	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Choline Chloride	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Sodium Chloride	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Dicalcium Phosphate	0.250	0.250	0.000	0.250	0.000	0.250	0.250	0.250
Titanium Dioxide	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500

[1]All diets were formulated to contain 20% CP.

[2] Animal by product blend. H. J. Baker & Bro., Little Rock, AR. ABB are a blend of various ingredients including the following: avian, ruminant, porcine, and marine animal proteins, steamed bone meal, Biolys-60, and DL methionine.

[3]Builder's Sand was used as an inert filler for dietary bulking.

[4]Vitamin and mineral premix included the following per kilogram of diet:
vitamin A (vitamin A acetate), 4,960 IU; vitamin D (cholecalciferol), 1,653 IU; vitamin E (dl-α-tocopherol acetate), 27 IU; menadione (menadione sodium bisulfate complex), 0.99 mg; vitamin B12 (cyanocobalamin), 0.015 mg; folic (folic acid), 0.8 mg; d-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 2.7 mg; d-biotin (biotin), 0.07 mg; pyridoxine (pyridoxine hydrochloride), 5.3 mg, Mn (manganous oxide), 90 mg; Zn (zinc oxide), 83 mg; Fe (iron sulfate monohydrate), 121 mg; Cu (copper sulfate pentahydrate), 12 mg; I (calcium iodate), 0.5 mg; and Se (sodium selenite), 0.3 mg.

Table 2.2 Analyzed total amino acid concentrations (%) of the experimental diets (as is)[1]

Nutrient	Semi-purified Diet							
	1	2	3	4	5	6	7	8
Essential AA[2]								
Met	0.24	0.23	0.23	0.24	0.24	0.25	0.46	0.29
Lys	0.95	1.10	0.94	0.89	0.86	0.96	1.00	1.07
Arg	1.32	1.31	1.31	1.30	1.26	1.40	1.25	1.53
Thr	0.74	0.71	0.67	0.64	0.57	0.62	0.72	0.71
Phe	0.78	0.75	0.73	0.69	0.65	0.67	0.77	0.76
Trp	0.11	0.12	0.12	0.13	0.11	0.11	0.06	0.10
Val	1.04	1.04	1.02	0.94	0.83	0.88	1.07	0.96
Ile	0.73	0.72	0.69	0.64	0.55	0.60	0.73	0.65
Leu	1.40	1.36	1.32	1.27	1.23	1.21	1.34	1.33
His	0.33	0.31	0.31	0.31	0.30	0.31	0.31	0.39
Nonessential AA								
Cys	0.45	0.42	0.39	0.29	0.20	0.25	0.53	0.19
Asp	1.47	1.45	1.44	1.41	1.34	1.43	1.30	1.60
Ser	1.04	0.98	0.89	0.83	0.65	0.73	1.25	0.86
Glu	2.29	2.29	2.25	2.24	2.27	2.28	2.15	2.62
Pro	1.64	1.66	1.63	1.60	1.65	1.74	1.55	1.92
Ala	1.26	1.27	1.30	1.33	1.44	1.46	1.10	1.64
Tyr	0.48	0.46	0.44	0.44	0.40	0.39	0.57	0.59
Total	19.42	19.49	19.09	18.82	18.53	19.43	19.08	22.15
CP	20.38	21.36	21.03	21.30	19.99	22.01	21.43	22.40
Titanium (ppm)	5000	3040	2920	2940	3120	3320	3030	3020

[1]Experiment Station Chemical Laboratories, University of Missouri-Columbia. Columbia, MO. Method 982.30 E(a, b, c; AOAC, 2000).
[2]Amino acid.

Table 2.3 Analyzed Amino Acid (%)[1] of Tested ABB[2]

Nutrient (%)	ABB							
	1	2	3	4	5	6	7	8
Lys	3.14	3.23	2.54	2.54	2.05	2.50	4.37	2.37
Met	0.71	0.65	0.64	0.64	0.57	0.59	1.39	0.58
Cys	1.40	1.07	1.00	0.80	0.48	0.61	1.81	0.46
Met + Cys	2.11	1.72	1.64	1.44	1.05	1.20	3.20	1.04
Thre	2.30	2.05	1.95	1.80	1.41	1.60	2.38	1.45
Phel	2.45	2.17	2.07	1.93	1.58	1.76	2.51	1.59
Try	0.36	0.39	0.35	0.34	0.28	0.33	0.36	0.30
Val	3.29	2.94	2.73	2.47	1.84	2.18	3.53	1.94
Iso	2.23	1.99	1.82	1.69	1.31	1.50	2.35	1.37
Leu	4.26	3.77	3.58	3.34	2.90	2.96	4.34	2.73
His	0.96	0.84	0.82	0.82	0.69	0.72	0.83	0.75
Arg	4.02	3.78	3.86	3.64	2.90	3.52	4.12	3.05

[1]CML+9. JAOAC 70:171-174, 1987. Experiment Station Chemical Laboratories, University of Missouri-Columbia. Columbia, MO.

[2]Animal by product blend. H. J. Baker & Bro., Little Rock, AR. ABB are a blend of various ingredients including the following: avian, ruminant, porcine, and marine animal proteins, steamed bone meal, Biolys-60, distillers dried grains and solubles, and DL methionine.

Table 2.4 Apparent ileal digestibility (%) of amino acids in animal by-product blend 1 (ABB1)[1] as affected by age of broilers

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	61.56[a]	66.49[a]	74.16[a]	58.96[a]	74.67[a]	66.10	66.99[a]	72.70[a]	68.74[a]	57.44[a]
42	54.69[b]	62.59[b]	69.22[b]	56.02[b]	69.47[b]	63.54	63.72[b]	68.99[b]	63.64[b]	54.94[b]
SEM[2]	0.948	0.779	1.110	1.016	0.813	2.216	0.914	0.929	0.971	1.18
P-Value	<0.0001	0.0033	0.0008	0.0135	<0.0001	0.2696	0.0037	0.0018	0.0002	0.0560
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	48.47	45.82	65.33[a]	64.01[a]	72.18[a]	71.56[a]	69.70[a]	65.47[a]		
42	46.31	43.31	61.39[b]	58.19[b]	67.09[b]	66.46[b]	65.68[b]	61.25[b]		
SEM	1.723	1.418	0.908	0.964	1.498	1.076	0.898	0.962		
P-Value	0.2340	0.1027	0.0010	<0.0001	<0.0001	0.0005	0.0008	0.0009		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB1 contains ruminant and avian animal protein products.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.5 Apparent ileal digestibility (%) of amino acids in animal by-product blend 2 (ABB2)[1] as affected by age of broilers

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	68.07	73.24	75.56	61.17	74.33	72.78	71.44	73.82	73.28	65.34[b]
42	68.10	73.47	73.21	63.12	73.68	75.47	71.86	74.47	73.45	68.68[a]
SEM[2]	1.498	1.172	1.409	1.776	1.404	1.957	1.540	1.347	1.464	1.417
P-Value[3]	0.9843	0.8481	0.1230	0.2959	0.6510	0.1555	0.7908	0.6394	0.9076	0.0380
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	49.83	49.64	61.56	66.50	66.88	71.57	67.46	66.09		
42	54.70	51.62	62.64	65.87	63.85	69.74	67.57	65.72		
SEM	2.528	2.033	1.791	1.557	2.104	1.708	1.519	1.674		
P-Value	0.0807	0.3497	0.5588	0.6941	0.1767	0.3058	0.9418	0.8274		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB2 contains ruminant and avian animal protein products, steamed bone meal, and Biolys 60.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.6 Apparent ileal digestibility (%) of amino acids in animal by-product blend 3 (ABB3)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	76.56	76.19	78.10	65.44	77.54	75.37	74.26	77.92	76.28	71.26
42	72.70	72.57	73.30	63.28	74.09	75.47	72.17	75.79	73.89	71.27
SEM[2]	2.154	2.192	2.392	3.148	2.307	1.690	2.528	2.181	2.270	2.349
P-Value[3]	0.119	0.104	0.068	0.206	0.161	0.951	0.424	0.349	0.313	0.997
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	47.73	59.02	62.28	71.86	70.11[a]	75.54[a]	71.13	70.42		
42	52.53	56.21	59.83	67.63	62.42[b]	70.26[b]	68.16	66.50		
SEM	3.960	3.023	3.400	2.421	2.591	2.345	2.694	2.472		
P-Value	0.249	0.371	0.466	0.106	0.012	0.044	0.292	0.103		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB3 contains ruminant, avian, and porcine animal protein products and Biolys 60.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.7 Apparent ileal digestibility (%) of amino acids in animal by-product blend 4 (ABB4)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	73.40	69.85	76.25	63.63	73.66	74.18	70.85	72.25	73.13	66.08
42	71.19	67.69	72.35	64.18	71.57	75.10	70.56	71.54	72.20	67.64
SEM[2]	1.535	1.698	1.864	2.170	1.700	1.271	1.859	1.507	1.611	1.476
P-Value[3]	0.1846	0.2351	0.0660	0.8069	0.2490	0.4877	0.8796	0.6485	0.5756	0.3183
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	31.18	53.82	63.89	68.69	67.53[a]	73.82[a]	67.88	67.36		
42	39.30	52.48	64.74	65.73	61.51[b]	69.55[b]	66.85	64.53		
SEM	3.055	1.957	2.181	1.642	1.761	1.893	1.610	1.670		
P-Value	0.0260	0.5124	0.7043	0.1051	0.0077	0.505	0.5378	0.1245		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB4 contains ruminant, avian, and porcine animal protein by products
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.8 Apparent ileal digestibility (%) of amino acids in animal by-product blend 5 (ABB5)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	69.47	65.25	70.92[a]	52.58	69.90	73.76	62.68	66.53	69.59	62.82[b]
42	69.55	65.58	65.90[b]	54.48	67.83	72.32	64.20	66.76	69.18	68.05[a]
SEM[1]	0.733	1.103	1.686	1.273	1.004	1.847	1.002	1.141	0.959	1.074
P-Value	0.9115	0.5555	0.0116	0.1708	0.0691	0.4532	0.1627	0.8265	0.6778	0.0007
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	32.49	50.44	47.28	64.62	63.27[a]	69.73	62.96	62.85		
42	28.26	52.53	47.16	63.04	57.77[b]	66.85	59.86	60.83		
SEM	3.029	1.366	1.961	1.293	2.015	1.744	1.801	1.349		
P-Value	0.0892	0.1644	0.9362	0.1842	0.0090	0.0598	0.2367	0.1299		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB5 contains ruminant and avian protein products and distillers dried grains and solubles.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.9 Apparent ileal digestibility (%) of amino acids in animal by-product blend 6 (ABB6)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	71.54	67.29	69.69	56.59	68.70	70.90	62.99	67.47	68.70	64.31[b]
42	70.39	66.57	66.55	57.38	67.16	72.86	63.77	68.00	68.79	68.00[a]
SEM[2]	0.771	1.218	1.686	1.633	1.129	1.955	1.199	1.034	1.116	1.074
P-Value[3]	0.1625	0.5681	0.0893	0.6376	0.1999	0.3378	0.5240	0.6153	0.9414	0.0056
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	32.49	54.79	50.67	64.63	61.70[a]	67.35	61.49	62.31		
42	28.26	55.32	52.61	62.99	55.79[b]	64.17	60.59	60.47		
SEM	3.029	1.366	1.961	1.293	2.015	1.744	1.801	1.349		
P-Value	0.0892	0.7082	0.3428	0.2309	0.0136	0.0954	0.6284	0.2011		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB6 contains ruminant and avian animal protein products.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

Table 2.10 Apparent ileal digestibility (%) of amino acids in animal by-product blend 7 (ABB7)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	86.84[a]	74.14[a]	74.92[a]	57.72[a]	74.17[a]	57.32	67.97[a]	74.38[a]	71.34[a]	68.82[a]
42	84.84[b]	70.00[b]	67.07[b]	52.58[b]	67.12[b]	55.78	60.71[b]	68.20[b]	63.80[b]	64.26[b]
SEM[2]	0.876	1.23	1.63	2.20	1.42	2.18	7.26	1.49	1.59	1.78
P-Value[3]	0.0485	0.0081	0.0010	0.0445	0.0008	0.4991	0.0035	0.0025	0.0010	0.0305
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	44.25[a]	40.74	63.33[a]	64.15[a]	63.53[a]	71.55[a]	73.23[a]	64.83[a]		
42	37.50[b]	39.00	56.68[b]	58.51[b]	58.10[b]	63.97[b]	69.81[b]	59.07[b]		
SEM	2.96	3.01	2.53	1.81	1.89	1.47	1.37	1.65		
P-Value	0.0489	0.5764	0.0272	0.0123	0.0182	0.0006	0.0340	0.0067		

[a,b]Values with different superscripts in a column differ significantly ($P \leq 0.05$)
[1]ABB7 contains porcine, avian, and marine animal protein products, Biolys 60, and DL methionine.
[2]Standard Error of Mean
[3]Significant level was set at $P \leq 0.05$

Table 2.11 Apparent ileal digestibility (%) of amino acids in animal by-product blend 8 (ABB8)[1] as affected by age of broilers.

Age (d)	Essential Amino Acids									
	Met	**Lys**	**Arg**	**Thr**	**Phe**	**Trp**	**Val**	**Ile**	**Leu**	**His**
21	73.23	70.04	73.64	63.38[b]	72.18	67.53[b]	66.80[b]	70.10[b]	71.77	71.68
42	74.11	71.58	73.98	66.71[a]	74.13	75.35[a]	73.63[a]	73.23[a]	73.77	73.17
SEM[2]	1.05	0.72	0.79	1.15	0.92	1.22	0.94	0.89	0.95	0.82
P-Value[3]	0.4201	0.0599	0.6664	0.0163	0.0610	0.0002	<0.0001	0.0054	0.0613	0.0996
Age (d)	**Nonessential Amino Acids**									
	Cys	**Asp**	**Ser**	**Glu**	**Pro**	**Ala**	**Tyr**	**Total**		
21	30.61[b]	56.89[b]	61.12[b]	67.40[b]	64.59	71.54	71.76[b]	66.24		
42	44.30[a]	60.12[a]	64.88[a]	69.77[a]	65.81	71.50	75.26[a]	68.02		
SEM	2.13	1.31	1.21	0.86	1.08	0.92	1.08	0.87		
P-Value	<0.0001	0.0329	0.0111	0.0207	0.2829	0.9706	0.0088	0.0675		

[a,b]Values with different superscripts in a column differ significantly (P≤0.05)
[1]ABB8 contains ruminants and avian animal protein products and steamed bone meal.
[2]Standard Error of Mean
[3]Significant level was set at P≤0.05

References

1. Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. 60:423-437.

2. Rostagno, H. S., J. M. R. Pupa, and M. Pack. 1995. Diet formulation for broilers based on total versus digestible amino acids. J. Appl. Poult. Res. 4:293-299.

3. Wang, X., and C. M. Parsons. 1998. Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acid basis. Poult. Sci. 77:1010-1015.

4. Fernandez, S. R., Y. Zhang, and C. M. Parsons. 1995. Dietary formulation with cottonseed meal on total amino acid versus a digestible amino acid basis. Poult. Sci. 74:1168-1179.

5. Kim, E. J., and A. Corzo. 2012. Interactive effects of age, sex, and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. Poult. Sci. 91:908-917.

6. Huang, K. H., V. Ravindran, X. Li, and W. L. Bryden. 2005. Influence of age on the apparent ileal digestibility of feed ingredients for broiler chickens. Br. Poult. Sci. 46:236-245.

7. H. J. Baker & Bro., Little Rock, AR.

8. NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

9. Aviagen. Huntsville, AL.

10. PECO Hatchery. Gordo, Alabama.

11. Alternative Design Manufacturing, Siloam Springs, AR.

12. Experiment Station Chemical Laboratories, University of Missouri-Columbia. Columbia, MO. (method 982.30 E(a, b, c; AOAC, 2000).

13. Experiment Station Chemical Laboratories, University of Missouri-Columbia. Columbia, MO. (method 990.08 (AOAC, 2000)

14. Experiment Station Chemical Laboratories, University of Missouri-Columbia.

15. SAS version 9.2. SAS Institute Inc. Cary, NC.

16. Kidd, M. T., A. Corzo, D. Hoehler, E. R. Miller, and W. A Dozier, III. 2005. Broiler responsiveness (Ross × 708) to diets varying in amino acid density. Poult. Sci. 84:1389-1396.

17. Shires, A., J. R. Thompson, B. V. Turner, P. M. Kennedy, and Y. K. Goh. 1987. Rate of passage of corn-canola meal and corn-soybean meal diets through the gastrointestinal tract of broiler and White Leghorn chickens. Poult. Sci. 66:289-298.

18. Parsons, C. M., F. Castanon, and Y. Han. 1997. Protein and amino acid quality of meat and bone meal. Poult. Sci. 76:361-368.

19. Nitsan, Z., G. Ben-Avraham, Z. Zoref, and I. Nir. 1991. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. Br. Poult. Sci. 32:515-523.

20. Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy and light strain chicks. Poult. Sci. 74:1622-1629.

21. Dror, Y., I. Nir, and Z. Nitsan. 1988. The relative growth of internal organs in light and heavy breeds. Br. Poult. Sci. 18:493-496.

22. Graber, G. and D. H. Baker. 1973. The essential nature of glycine and proline for growing chickens. Poult. Sci. 52:892-896.

23. Tsiagbe, V. K., M. E. Cook, A. E. Harper, and M. L. Sunde. 1987. Efficacy of Cysteine in replacing methionine in the immune responses of broiler chicks. Poult. Sci. 66:1136-1146.

CHAPTER III

EFFECTS OF VARIOUS FEED ADDITIVE STRATEGIES ON BROILERS GIVEN 10× LIVE COCCIDIOSIS VACCINE

Summary

Various feed additives have been used to combat coccidiosis infections; however, there is still a need for new products and/or application strategies to be investigated, especially those used in conjunction with live coccidiosis vaccines (**LCV**). Therefore, the objective of the current study was to determine the effects of the inclusion strategies of several commercially available feed additives on broilers sprayed with 10× LCV. Additives of interest for this particular study include virginiamycin (**VM**), bacitracin methylene disalicylate (**BMD**), salinomycin (**SAL**); as well as two alternative products, a probiotic (**PRO**) and an herb and essential oil blend (**EB**). All treatments (**Trt**) received 10× LCV, excluding Trt 1, the unmedicated negative control (**NC**). Treatment 2, the positive control (**PC**), was also unmedicated but birds were sprayed with 10× LCV. Over the course of the study birds that were sprayed with LCV and fed diets containing VM with an addition of SAL during d 14-28 only demonstrated increased BW on d 14, 28, 42, and 49 when compared to the Trt 2 (NC); cumulative growth performance also demonstrated larger d 0-49 BWG in birds fed Trt 3 (VM+SAL). Gastrointestinal organ measurements and morphology were not significantly affected by Trt (d 17). Utilizing Trt 3 (VM+SAL) and 8 (BMD+SAL) demonstrated the ability to reduce lesion scores

caused by *Eimeria acervulina* at d 17. Ileal digesta viscosity was significantly increased in birds when fed Trt 3 (VM+SAL).

Key words: antibiotics, anticoccidials, antibiotic alternative products, live coccidiosis vaccine, bird performance

Description of Problem

Coccidiosis is considered to be the largest economical liability in the poultry industry attributing to a 1 to 3 billion dollar loss per year on a global scale [1, 2]. These economic losses stem from overall decreased live performance, including increased FCR and mortality; as well as increased costs associated with preventative controls [3]. Some of these preventative controls include the use of synthetic drugs, ionophoric anticoccidials, and modified vaccines that have been in use since the late 1940's [4, 5]. Ionophores and vaccines are still commonly used today with great success and have demonstrated improved live performance [6-9] and also decreased lesion scores [6-8]. Live coccidiosis vaccines (**LCV**) sprayed at the hatchery can be used in rotation with anticoccidials to decrease development of pathogenic resistance. When used in rotation programs, LCV help restore sensitivity to anticoccidials by infecting chicks with drug-sensitive oocysts that produce progeny that are more likely sensitive to anticoccidials [10]. Therefore, it is important to not use anticoccidials in feed during the first two weeks of growth to allow oocysts to recycle from the litter and subsequently provoke an immune response once ingested. However, if the LCV does not utilize drug sensitive oocysts, LCV can lead to late cycling oocysts excreted into the litter. These anticoccidials, specifically ionophores, help reduce *Eimeria* parasite infections by acting

as a “bridge” or “shuttle” to allow for free ion exchange disrupting *Eimeria* cell membranes [11].

Today many poultry integrators are still using antibiotics and anticoccidials to medicate diets; however, due to the concerns with toxicity and consumer demand, synthetic drug use in the poultry industry has raised concern [4, 12]. Additionally, prolonged use of antibiotics and anticoccidials has also led to the emergence of *Eimeria* strains that are becoming increasingly resistant to the drugs; therefore, integrators are interested in incorporating new alternative products to help decrease resistance issues as well as provide a more natural product for the consumer [13].

In addition to the previously mentioned controls, alternative feed additives have been found to help control coccidiosis including probiotics [14] and herb and essential oil blends [15, 16]. Research has shown that these alternative products added to feed maintain or improve broiler performance [14-17]. More recent trials have found that probiotics can be beneficial to broiler live performance [8, 9] as well as decrease lesion scores [7-9]. Herb and essential oil blends have also shown positive results in reducing necrotic enteritis, improve intestinal morphology, and stimulate the immune system [16, 18]. These contributions can lead to increased live performance of broilers by maintaining a healthy gut environment.

While extensive research has been conducted on these products individually and in some specific combinations, our objective was to determine inclusion strategies utilized to combat late cycling of coccidia with use of LCV, reestablish drug sensitivity, or provide alternative methods of medication for integrators progressing towards antibiotic free management. Therefore, the current study investigated industry applicable

inclusion strategies during d 0-49 and d 14-28 (select treatments) to demonstrate the effects of various antibiotics, an anticoccidial, a probiotic, and an herb and essential oil blend on live performance and gut characteristics of broiler chicks sprayed with 10× LCV.

Materials and Methods

Diet Formulations

Diet formulations were proprietary and initially batched without feed additives at a commercial feed mill [19]. Basal diets were corn-soybean meal based and also contained meat and bone meal. The analysis of feed samples for each diet can be found in Table 3.1. Starter-phase diets were fed from d 0-14 of age in the form of crumbles. Grower, finisher, and withdrawal diets were fed in the form of pellets from 14-28, 28-42, and 42-49 day of age, respectively. Prior to pelleting, feed additives were included into each respective treatment in a small mixer with approximately 11 kg of the basal diet. After 5 minutes of mixing the small basal batch was then added to the large basal batch and mixed for an additional 4 minutes before pelleting.

Description of Dietary Treatments

Treatment 1 birds were fed diets lacking feed additives, thus noted as the negative control (NC) hereafter. Treatment 2 birds were also fed diets lacking feed additives, but were sprayed with 10× LCV [20] and henceforth reported as the positive control (PC). Birds fed the remaining Trt, 3-8, were sprayed with the 10× LCV as well.

Briefly, feed additives utilized included virginiamycin 15g/t (VM), bacitracin methylene disalicylate 50g/t (BMD), salinomycin 40g/t (SAL), a *Bacillus subtilis* based

probiotic (PRO) 226.8g/t, and an herb and essential oil blend 127g/t (EB) [21-24]. All feed additives were included at the manufacturer's recommendations. However, it should be noted again, SAL was only included into diets on d 14-28 for Trt 3 and Trt 8 (henceforth noted as VM+SAL and BMD+SAL, respectively) and BMD inclusion was increased during d 14-28 for Trt 7; increased BMD inclusion will be noted as BMD. Table 3.2 is provided with the intention of explaining treatments and feed additive inclusions.

Bird Husbandry

This study was approved by the Mississippi State University Institutional Animal Care and Use Committee. The experimental house was solid-walled and located at Mississippi State University Poultry Research Center. One thousand three hundred and forty four day-old Ross × Ross 708 broiler chicks [25] were used for this experiment. Chicks were obtained from a commercial hatchery [26] and vaccinated for Marek's disease, Newcastle disease, infectious bronchitis, as well as *Eimeria spp.* (LCV) [20] at ten times the recommended dose, while 168 chicks did not receive the *Eimeria spp.* vaccination. Chicks were feather-sexed so that seven males and seven females were randomly allocated to one of 96 floor pens (14 birds/pen) with a stocking density of 0.08 m^2/bird. Each floor pen was equipped with a hanging commercial feeder, a nipple drinker line (3 nipple drinkers/pen), and top dressed with used litter from a commercial research house.

Ambient temperature started at 32°C and decreased incrementally until 16.1°C was reached on d 49. Lighting was adjusted so that birds received 32.3 lux from 0 to 7 d with 23 hours of light and 1 hour of dark. On d 10, birds received 19L:5D and light

intensity decreased incrementally until 2.7 lux was reached on 21; this lighting schedule and intensity was maintained throughout the remaining experiment.

Gastrointestinal Sampling

Sampling Procedure

On day 17, two birds (one proposed male and one female) were removed and weighed from half of the replicate pens (48, 6 replications/treatment) for organ and gastrointestinal tract sampling, as well as histology slide creation. Males and females were chosen based on phenotypic characteristics; sex was confirmed upon sampling.

Tract and Organ Measurement

The duodenum, jejunum, ileum (meckel's diverticulum to ileo-cecal junction), and ceca were removed and individually weighed without the digesta contents. After digesta was removed, organ length was measured in centimeters. The weight of the duodenum, jejunum, ileum, and ceca were also recorded as a percentage of live BW.

Morphology

A small section (38.1mm) of the duodenum, jejunum, and ileum was obtained from each bird that was used for GI tract measurements. The small piece of tissue was excised from the middle of each respective tract and then stored in a 20ml white capped scintillation vial containing 10 ml of 10% buffered formalin [27]. Tissue samples were dehydrated and embedded in paraffin. One slide was prepared from each paraffin block and stained with alcian blue [28]. Two sections from the tissue sample were placed on every slide for staining. Using Motic Images Plus 2.0 software [29], digital pictures were then taken of each slide at 4x and 40x with a Swift M10 Series microscope [30] and

digital camera attachment [31]. Villus height, crypt depth, goblet cell count were determined using ImageJ software [32]. These procedures follow similar methods used by Wang et al. [33].

Lesion Scoring

On day 17, two birds (one proposed male and female; sex confirmed upon sampling) were removed and weighed from the second half of the replicate pens (48, 6 replications/treatment) for lesion scoring. After cervical dislocation the digestive tract was removed. Lesion scoring was performed by poultry veterinarians blinded by treatment using methods adapted from Johnson and Reid [34]. Lesion scores were scored upon severity, with 1 being mild and 4 being severe. The duodenum was opened using a scalpel and lesions were scored if present as mild, small white nodules (#1) or in the more severe cases larger dark nodules (#4). This process was repeated for the jejunum, ileum, and cecum. Lesions scores were then recorded and average lesion score per treatment was determined.

Ileal Digesta Viscosity

At day 17, one proposed male (sex was confirmed upon sampling) was removed from every pen. The bird was weighed and then killed via cervical dislocation. The contents of the ileum (meckel's diverticulum to ileo-cecal junction) were removed and individually collected in a plastic tube. Tubes were then placed on ice until all samples were collected, then transported to the Department of Poultry Science at Mississippi State University for viscosity determination.

First, individual digesta contents were homogenized in their respective tube. Then, two 1.5 ml Eppendorf tubes were filled with the digesta and centrifuged at 9,500 × g for 5 minutes. Next, 250 µL of the supernatant was removed from each tube to create a pooled sample of 0.5mL supernatant and transferred to the Brookfield digital viscometer [35]. Viscosity was determined by running at 12 RPM for 60 s. at a shear rate of 90 s^{-1} at 25º C. Procedures for this sampling were adapted from Corey and cohorts [36].

Bird Performance

Live performance measured variables included Average (**AVG**) BW and body weight gain (**BWG**), Pen feed intake (**FI**) and FCR for d 0-14, 14-21, 14-28, 28-42, and 42-49, as well as cumulative d 0-49. Mortality was collected daily and saved for necropsy, as well as sex determination. Mortality weights were used to calculate FCR.

Statistical Analysis

Each of the 8 treatments had 12 replicate pens and treatments were assigned as a Randomized Complete Block and blocked by location. Statistical analyses for this project were performed using the GLM procedure in SAS [37]. The experimental unit was considered to be one floor pen with 14 birds in each. Multiple comparisons were made with all treatment means using Fisher's Least Significant Difference test after one-way Analysis of Variance (**ANOVA**) was determined. For tract and organ measurements as well as morphology, 2 birds from each pen from blocks 1-6 (6 replications/Trt) were utilized. Lesion scoring utilized 2 birds/pen from the second half of blocks, 7-12 (6 replications/Trt). Finally, ileal digesta viscosity used 1 birds/pen from each block (12 replications/Trt). Alpha was set at $P \leq 0.05$.

Results and Discussion

Gastrointestinal Tract Measurements

Feed additive strategy demonstrated no effect on duodenum, jejunum, ileum, and ceca weights, % BW, and length (P>0.05; Table 3.3). Additionally, no significant differences were found for Avg. villus height, crypt depth, and goblet cell count of the duodenum, jejunum, and ileum (P>0.05; Table 3.4).

Lesion Score

Before discussing lesion score data it should be noted that diets containing d 14-28 feed additive interventions (Trt 3, 7, and 8) were not fed for an extended period of time before sampling on d 17. These interventions were utilized to reduce the negative effects accompanied by LCV and recycling of oocysts. In the study lesion scores of *E. maxima* and *E. tenella* were not significantly affected by the dietary treatments (P>0.05; Table 3.5). However, birds that were fed Trt 3 (VM+SAL) and Trt 8 (BMD+SAL) demonstrated significantly decreased Avg. lesion score caused by *E. acervulina* (P=0.017) when compared to birds fed Trt 2 (PC). These results are consistent with previous research in which addition of SAL was reported to decrease lesion scores caused by *E. acervulina* [6, 7]. We believe the reduction in lesion score was attributed to the inclusion of SAL, as ionophores combat *Eimeria* parasites by allowing bidirectional exchange of ions, thus disrupting the cell membranes. This can also be noted by the differing results of Trt 4 (VM) and Trt 3 (VM+SAL) (Table 3.5). Due to the inclusion intervention of increasing the dose of BMD during d 0-14 for Trt 7 (BMD), the comparison to Trt 8 (BMD+SAL) cannot be clearly defined (Table 3.5). It must be noted that lesion scores were reported as an average of two birds from each replicate group.

Overall, lesion scores found were most commonly scored as 1 (mild) in birds sampled. Obtained results indicate that the 10× LCV and used litter served only as a slight or mild challenge.

Ileal Digesta Viscosity

Birds fed Trt 3 (VM+SAL) demonstrated significantly increased digesta viscosity compared to Trt 2 (PC) ($P<0.0001$; Table 3.6). This viscosity is relatively high compared to other viscosity measurements obtained, but due to cumulative d 0-49 performance data demonstrating Trt 3 as one of the most beneficial Trt in regards to BWG and lesion score, this could be the optimal digesta viscosity. However, Trt 5 (BMD+PRO) and Trt 7 (BMD) yielded similar results in regards to d 0-49 BWG yet significantly lower ileal digesta viscosity. To our knowledge the effects of various feed antibiotics, anticoccidials, and alternative products on digesta viscosity are not clearly defined, however, the increased viscosity could have allowed for decreased rate of passage and thus increasing nutrient absorption that might be correlated to live performance benefits observed in Trt 3 [38].

Bird Performance

All live performance results can be found in Table 3.7. Pen feed intake and mortality % will not be discussed due to the non-significance of FI and the cumulatively low mortality % observed during the experiment ($P>0.05$; Table 3.7).

BW Effects

Treatment 3 (VM+SAL) demonstrated the most consistent effects on the measured variable Avg. BW/bird ($P<0.05$) on d 14, 28, 42, and 49. On each of these

days, birds demonstrated significantly increased BW when compared to the positive control diet, Trt 2 (PC). In addition to Trt 3 (VM+SAL), Trt 5 (BMD+PRO) and 7 (BMD) demonstrated similar BW improvements on each of these specific days excluding Trt 7 on d 42. Similar to the current data, previous research has shown that the addition of SAL to diets fed to 42 d old broilers demonstrated improved BW when compared to birds receiving NA and a coccidiosis challenge [8]. Additionally, VM has been reported to increase BW at d 49 when compared to diets without antibiotics [39]. While VM has traditionally been used as a growth promoting antibiotic the addition of SAL at d 14-28 may have provided anticoccidial effects to the diet and therefore lead to this significant improvement in BW observed over the course of the experiment. The similarities between Trt 3 (VM+SAL) and Trt 5 (BMD+PRO) and Trt 7 (BMD) help demonstrate that more than one inclusion strategy may be applicable to the industry. In consideration of the efficacy of BMD, d 49 BW were significantly increased in birds when diets were supplemented with BMD compared to diets lacking the additive [39]. Improvements in BW provided by supplementation of PRO have also been reported in several studies when compared to control diets [8, 40]. The benefits observed while supplementing with PRO could be due to probiotic bacteria's competitive exclusion characteristics for attachments sites on the intestinal epithelial cells [7, 8]; however, this cannot be confirmed by this current study.

An additional measurement period was added, d 14-21, due to the prepatent period of 4 to 5 days following oral infection and maximum oocyst shedding 6 to 9 days following [41]. This period was also included to determine if inclusion strategies could reduce negative effects observed by recycling oocysts. Additionally, this period can shed

light on the extent of our coccidiosis challenge provided via 10× LCV. Average d 21 BW was not significantly affected by the treatments; however, a trend was established (P=0.080; Table 3.7). Birds that received Trt 2 (PC), had a tendency to have the lowest BW when compared to the other treatments on d 21, including the negative control, Trt 1 (NC; P=0.080). Treatment 3 (VM+SAL), Trt 5 (PRO+BMD), and Trt 7 (BMD) demonstrated numerically increased d 21 BW vs. Trt 2 (PC), which corresponds with BW improvements at d 14, 28, 42, and 49 (P<0.05; Table 3.7). Considering d 49 BW also demonstrated birds fed Trt 2 (PC) had the significantly lowest BW at the end of the study (P=0.0013; Table 3.7), we can speculate that some level of challenge was provided via 10× LCV. Birds fed Trt 4 (EB) also numerically demonstrated the lowest d 21 BW; however, these birds demonstrated low BW throughout the remainder of the trial, providing speculation the EB was not effective in improving or maintaining growth performance with 10× LCV.

In regard to BWG during each specific phase of feeding and cumulative, only d 0-14, 14-28, and 0-49 were significantly affected by treatments. During d 0-14 and d 14-28 birds that were fed Trt 3 (VM+SAL) demonstrated improved BWG (P=0.018 and P<0.0001, respectively; Table 3.7) vs. Trt 2 (PC), which was to be expected due to their superior d 14 and d 28 BW previously mentioned. Again Trt 5 (BMD+PRO) and Trt 7 (BMD) demonstrated similar BWG performance to Trt 3 (VM+SAL) during d 0-14 and d 14-28. Treatment 4 (VM) and Trt 6 (EB) should be noted as performing similarly to Trt 3 (VM+SAL) in regards to d 0-14 BWG, however, this performance was not observed in the later periods of growth and subsequently decreased through the study. Ultimately, Trt 3 (VM+SAL) and Trt 7 (BMD) proved to have superior BWG (P=0.018; Table 3.7)

during the cumulative study, d 0-49, when compared to Trt 2 (PC) and the remaining Trt. We hypothesize that the inclusion interventions utilized with the addition of SAL to VM (Trt 3) and increased dose of BMD during d 14-28 may have helped reduce the negative effects of recycling oocysts and therefore, consequently improved BW and BWG.

FCR Effects

Feed conversion ratios were significantly effects by the dietary treatments during the periods of d 0-14 and 14-28. During d 0-14 birds fed Trt 4 (VM) or Trt 7 (BMD*) demonstrated the lowest FCR when compared to birds fed Trt 2 (PC) ($P<0.0001$; Table 3.7). It should be noted, during this period Trt 3 (VM+SAL), 5 (PR+BMD), 6 (EB), and 8 (BMD+SAL) demonstrated similar improvements and can be noted by the difference of 1 point in FCR (Table 3.7). The improvements made to FCR can be demonstrated in previous research that utilized inclusions of VM or BMD into diets and compared the performance of birds fed unmedicated diets [39, 42, 43]. This may not be surprising as VM and BMD use has been prevalent in the poultry industry for many years [44].

During d 14-28 birds that were fed Trt 8 (BMD+SAL) demonstrated improvements to FCR when compared to birds fed Trt 2 (PC) ($P=0.002$; Table 3.7). It is interesting to note that at this time period Trt 3 (VM+SAL) performed statistically similar to Trt 8. During this time period the common feed additive in both diets was SAL. Comparing Trt 3 (VM+SAL) to Trt 4 (VM) we can see that Trt 4 is significantly higher than Trt 3. Thus in this case, SAL appears to have had a beneficial role on FCR during d 14-28. However, the same type of comparison cannot be made for Trt 8 (BMD+SAL) and Trt 7 (BMD) because both Trt utilized a change of inclusion strategy during d 14-28.

In regards to SAL, improvements in FCR have been noted in d 15-21 [7] and d 15-28 [8] during previous studies

Overview

Current results suggest that integrators interested in utilizing medicated diets that may help reduce late cycling of *Eimeria* oocysts associated with LCV application or help progress to an antibiotic feed management can adopt inclusion strategies of VM and SAL during the d 14-28 phase (Trt 3), PRO and BMD (Trt 5) throughout, and BMD with an increased dose during d 14-28 (Trt 7). However, as consumers push for decreased antibiotic and anticoccidial use in commercial production it could potentially be beneficial to switch to alternative feed additives such as a probiotic fed alone, etc. Furthermore, given that some of these feed additives were included into diets alone or in combination, further research is warranted during a phase feeding grow-to better determine their effects on broiler live performance and intestinal characteristics.

Conclusions and Applications

1. Day 17 gastrointestinal tract and organ measurements were not affected by dietary Trt, whereas, d 17 *E. acervulina* lesion scores were reduced in birds fed VM+SAL (Trt 3) and ileal digesta viscosity was increased due to the same dietary Trt.
2. Inclusion interventions accomplished through the addition of 50g/t SAL to diets containing 15g/t VM (Trt 3) and increased supplementation of 200g/t BMD (Trt 7) during the feeding phase of d 14-28 demonstrated a 4.2 and

3.2% increase in final d 49 BW compared to birds sprayed with 10× LCV and fed unmedicated diets (Trt 2).

3. Diets containing 226.8g/t PRO and 50g/t BMD in each phase of the diet demonstrated an increase of 2.5% for final d 49 BW when compared to birds sprayed with 10× LCV and fed unmedicated diets (Trt 2).

Table 3.1 Analysis of Basal Diets

Nutrient[1]	Phase of Growth			
	Starter (d0-14)	Grower (d14-28)	Finisher (d28-42)	Withdrawal (d42-49)
Lysine	1.23	1.25	1.10	1.08
Methionine	0.34	0.30	0.28	0.29
Cysteine	0.32	0.30	0.29	0.31
Threonine	0.75	0.74	0.72	0.70
Isoleucine	0.77	0.74	0.66	0.67
Leucine	1.64	1.58	1.51	1.53
Proline	1.33	1.33	1.36	1.36
Glycine	1.00	1.04	1.13	1.06
Alanine	1.04	1.04	1.05	1.04
Valine	0.87	0.85	0.85	0.88
Aspartic Acid	1.91	1.82	1.63	1.62
Glutamic Acid	3.22	3.09	2.87	2.88
Crude Protein %[2]	20.79	19.42	17.51	18.12
Gross Energy (kcal/kg)[3]	3930.12	3901.84	3910.39	3923.77

[1]CML+9. JAOAC 70:171-174, 1987. Agricultural Experiment Station University of Missouri-Columbia.
[2]Determined by LECO 528 Nitrogen Determinator at Mississippi State University
[3]Determined by Parr Bomb Calorimeter at Mississippi State University

Table 3.2 Dietary Treatment Outline

Treatment	LCV[1] (+ or -)[2]	Starter d0-14	Grower d14-28	Finisher d28-42	Withdrawal d42-49
1	-	NA[3]	NA	NA	NA
2	+	NA	NA	NA	NA
3	+	VM[4] (20g/t)	VM(20g/t) + SAL[5] (40g/t)	VM (20g/t)	VM (20g/t)
4	+	VM (20g/t)	VM (20g/t)	VM (20g/t)	VM (20g/t)
5	+	PRO[6] (226.80g/t) + BMD[7] (50g/t)	PRO (226.80g/t) + BMD (50g/t)	PRO (226.80g/t) + BMD (50g/t)	PRO (226.80g/t) + BMD (50g/t)
6	+	EB[8] (127g/t)	EB (127g/t)	EB (127g/t)	EB (127g/t)
7	+	BMD (50g/t)	BMD (200g/t)	BMD (50g/t)	BMD (50g/t)
8	+	BMD (50g/t)	BMD (50g/t) + SAL (40g/t)	BMD (50g/t)	BMD (50g/t)

[1]Live Coccidiosis Vaccine. [20]
[2] '+' signifies birds were sprayed with 10× LCV at hatchery; '–' signifies birds were not sprayed with 10× LCV at hatchery.
[3]No additives were included to basal diet.
[4]Virginiamycin. [21]
[5]Salinomycin. [22]
[6]Probiotic. [23]
[7]Bacitracin Methylene Disalicyalte. [22]
[8]Essential herb/oil blend. [24]

Table 3.3 Effects of Dietary Treatments on Gastrointestinal Tract and Organ Measurements

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Duodenum											
Weight (g)	7.59	7.9	7.73	8.08	7.58	7.75	8.19	7.53	0.354	-	0.822
% BW	1.19	1.26	1.21	1.31	1.19	1.19	1.25	1.19	0.049	-	0.423
Length (cm)	21	21.6	21.1	21.9	20.8	21.3	22.2	20.7	0.730	-	0.708
Jejunum											
Weight (g)	12	12.2	11.8	13	12.5	12.7	12.5	12.1	0.369	-	0.410
% BW	1.9	1.95	1.86	2.13	1.97	1.96	1.91	1.93	0.055	-	0.054
Length (cm)	47.5	47	45.5	44	47.3	47.9	45.3	45.8	2.026	-	0.0375
Ileum											
Weight (g)	7.95	8.06	7.68	8.35	8.32	8.07	8.29	8.12	0.350	-	0.806
% BW	1.25	1.28	1.21	1.36	1.31	1.24	1.26	1.28	0.048	-	0.473
Length (cm)	48	46.9	44.2	45.8	47.8	47.4	45.4	45.5	1.658	-	0.601
Ceca											
Weight (g)	2.19	2.01	2.24	2.43	2.26	2.26	2.29	2.34	0.123	-	0.596
% BW	0.348	0.324	0.355	0.395	0.357	0.349	0.348	0.374	0.019	-	0.448
Length (cm)	9.12	9.05	9.06	9.13	9.3	9.93	9.14	9.4	0.625	-	0.625

a-dMeans within the same row with no common superscript are significantly different

1Trt 1 Negative Control (No Additives), Trt 2 Positive Control (Live Coccidiosis Vaccine+No Additives), Trt 3 VM+SAL (Live Coccidiosis Vaccine+Virginiamycin+(Salinomycin d 14-28)), Trt 4 VM (Live Coccidiosis Vaccine+Virginiamycin), Trt 5 PRO+BMD (Live Coccidiosis Vaccine+Probiotic+Bacitracin Methylene Disalicylate), Trt 6 EB (Live Coccidiosis Vaccine+Essential Oil Blend), Trt 7 BMD (Live Coccidiosis Vaccine+BMD (Increased dose d 14-28)), Trt 8 BMD+SAL (LCV+Bacitracin Methylene Disalicylate+(Salinomycin d 14-28)); One proposed male and one female (based on phenotypical characteristics) were sampled from half the blocks (1-6) (6 replications/Trt).

2Standard Error of the Mean.

3Fisher's Least Significant Difference.

3Significance level set at P≤0.05.

Table 3.4 Effects of Dietary Treatments on Duodenum, Jejunum, and Ileum Morphology

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Duodenum											
Avg. Villus Ht.	1644.80	1735.09	1638.77	1724.99	1737.16	1831.13	1592.17	1685.74	64.77	-	0.266
Avg. Crypt Depth	191.1	228.28	199.74	220.55	203.58	220.58	188.4	199.3	13.95	-	0.366
Avg. Goblet Cell Count	9.5	10.083	10	10	11.083	10.5	8.333	10.917	1.22	-	0.826
Jejunum											
Avg. Villus Ht.	813.01	803.79	791.18	788.51	807.56	810.69	813.67	796.73	39.90	-	0.999
Avg. Crypt Depth	149.85	152.76	166.84	174.63	152.36	150.04	161.49	134.76	13.47	-	0.578
Avg. Goblet Cell Count	16.167	15.417	14.417	13.917	13.333	14.25	15.75	15.25	1.47	-	0.867
Ileum											
Avg. Villus Ht.	494.49	452.05	435.87	499.66	471.4	502.62	407.2	457.07	22.96	-	0.632
Avg. Crypt Depth	139.1	154.3	142.28	138.91	131.21	156.53	127.12	141.86	9.42	-	0.355
Avg. Goblet Cell Count	12.333	11.5	9.25	11.167	12.667	12.167	11.75	10.583	1.07	-	0.411

[a-d]Means within the same row with no common superscript are significantly different

[1]Trt 1 Negative Control (No Additives), Trt 2 Positive Control (Live Coccidiosis Vaccine+No Additives), Trt 3 VM+SAL (Live Coccidiosis Vaccine+Virginiamycin+(Salinomycin d 14-28)), Trt 4 VM (Live Coccidiosis Vaccine+Virginiamycin), Trt 5 PRO+BMD (Live Coccidiosis Vaccine+Probiotic+Bacitracin Methylene Disalicylate), Trt 6 EB (Live Coccidiosis Vaccine+Essential Oil Blend), Trt 7 BMD (Live Coccidiosis Vaccine+BMD (Increased dose d 14-28)), Trt 8 BMD+SAL (LCV+Bacitracin Methylene Disalicylate+(Salinomycin d 14-28)); Birds utilized were the same birds removed from half the blocks (1-6) (6 replications/trt) used to determine organ measurements.

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at $P \leq 0.05$.

Table 3.5 Effects of Dietary Treatments on Avg. Intestinal Lesion Score

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
***E. acervulina*[5]**	0.17^{bc}	0.5^{ab}	0.00^{c}	0.42^{ab}	0.33^{abc}	0.67^{a}	0.25^{bc}	0.00^{c}	0.139	0.398	0.017
***E. maxima* (gross)[6]**	0.00	0.00	0.00	0.00	0.00	0.08	0.08	0.00	0.038	-	0.448
***E. maxima* (micro)[7]**	0.25	0.25	0.25	0.33	0.33	0.17	0.17	0.08	0.14	-	0.91
***E. tenella*[8]**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-

[a-d]Means within same column with no common superscript are significantly different

[1]Trt 1 Negative Control (No Additives), Trt 2 Positive Control (Live Coccidiosis Vaccine+No Additives), Trt 3 VM+SAL (Live Coccidiosis Vaccine+Virginiamycin+(Salinomycin d 14-28)), Trt 4 VM (Live Coccidiosis Vaccine+Virginiamycin), Trt 5 PRO+BMD (Live Coccidiosis Vaccine+Probiotic+Bacitracin Methylene Disalicylate), Trt 6 EB (Live Coccidiosis Vaccine+Essential Oil Blend), Trt 7 BMD (Live Coccidiosis Vaccine+BMD (Increased dose d 14-28)), Trt 8 BMD+SAL (LCV+Bacitracin Methylene Disalicylate+(Salinomycin d 14-28)); One proposed male and one female (based on phenotypical characteristics) were sampled from half the blocks (7-12) (6 replications/Trt).

[4]Significance level set at $P \leq 0.05$.

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[5]*Eimeria acervulina* is commonly found in the duodenum and noted by white or grayish lesions.

[6]*Eimeria maxima* lesions can be found in the jejunum and can be confirmed by thickening of intestine and use of microscope.

[7]*Eimeria maxima* micro lesions were confirmed using a microscope due to the diagnostic nature of large oocysts.

[8]*Eimeria tenella* is commonly found in the ceca and noted by blood and necrotic material in ceca.

Table 3.6 Effects of Dietary Treatments on Ileal Digesta Viscosity

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Viscosity[5]	2.65^{bc}	2.53^{cd}	3.06^{a}	2.32^{d}	2.57^{c}	2.80^{b}	2.68^{bc}	2.54^{cd}	0.078	0.220	<0.0001

[a-d]Means within same column with no common superscript are significantly different

[1]Trt 1 Negative Control (No Additives), Trt 2 Positive Control (Live Coccidiosis Vaccine+No Additives), Trt 3 VM+SAL (Live Coccidiosis Vaccine+Virginiamycin+(Salinomycin d 14-28)), Trt 4 VM (Live Coccidiosis Vaccine+Virginiamycin), Trt 5 PRO+BMD (Live Coccidiosis Vaccine+Probiotic+Bacitracin Methylene Disalicylate), Trt 6 EB (Live Coccidiosis Vaccine+Essential Oil Blend), Trt 7 BMD (Live Coccidiosis Vaccine+BMD (Increased dose d 14-28)), Trt 8 BMD+SAL (LCV+Bacitracin Methylene Disalicylate+(Salinomycin d 14-28)); 1 proposed male (based on phenotypical characteristics was removed from all blocks (1-12) (12 replications/Trt).

[2] Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at $P \leq 0.05$.

[5] Unit of measure: Centipoise.

Table 3.7 Effects of Dietary Treatments on Live Broiler Performance

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Avg. BW (kg)											
d 14	0.429^{bcd}	0.421^{d}	0.441^{a}	0.436^{abc}	0.436^{abc}	0.438^{ab}	0.438^{ab}	0.425^{cd}	0.0044	0.0132	0.011
d 21	0.912	0.885	0.935	0.885	0.912	0.926	0.921	0.903	0.0141	-	0.080
d 28	1.343^{cde}	1.307^{e}	1.416^{a}	1.325^{de}	1.393^{ab}	1.357^{bcd}	1.375^{abc}	1.379^{abc}	0.0159	0.0477	<0.0001
d 42	2.740^{bcd}	2.718^{cd}	2.854^{a}	2.718^{d}	2.809^{ab}	2.768^{bcd}	2.786^{bc}	2.777^{bcd}	0.0284	0.0852	0.0008
d 49	3.403^{bc}	3.394^{c}	3.539^{a}	3.398^{bc}	3.480^{ab}	3.403^{bc}	3.503^{a}	3.466^{abc}	0.0341	0.102	0.0013
Avg. BWG[5] (kg)											
d 0-14	0.388^{bcd}	0.382^{d}	0.403^{a}	0.395^{abc}	0.395^{abc}	0.397^{ab}	0.397^{ab}	0.384^{cd}	0.0045	0.0135	0.018
d 14-21	0.364	0.348	0.373	0.328	0.361	0.366	0.367	0.354	0.0131	-	0.335
d 14-28	0.799^{bcd}	0.771^{d}	0.862^{a}	0.767^{d}	0.839^{ab}	0.794^{cd}	0.821^{abc}	0.835^{abc}	0.0146	0.0438	<0.0001
d 28-42	1.416	1.411	1.452	1.397	1.407	1.420	1.416	1.379	0.0238	-	0.652
d 42-49	0.667	0.667	0.649	0.653	0.667	0.626	0.721	0.658	0.0201	-	0.122
d 0-49	3.407^{bc}	3.339^{c}	3.534^{a}	3.353^{c}	3.426^{abc}	3.376^{bc}	3.475^{ab}	3.389^{bc}	0.0402	0.1206	0.018
FCR											
d 0-14	1.32^{b}	1.35^{a}	1.30^{bc}	1.29^{c}	1.30^{bc}	1.30^{bc}	1.29^{c}	1.30^{bc}	0.0083	0.024	<0.0001
d 14-21	1.39	1.39	1.35	1.44	1.40	1.35	1.39	1.38	0.0402	-	0.867
d 14-28	1.66^{a}	1.66^{ab}	1.62^{cd}	1.66^{a}	1.63^{bcd}	1.64^{abcd}	1.64^{abcd}	1.61^{d}	0.0114	0.032	0.002
d 28-42	1.91	1.91	1.91	1.92	1.96	1.92	1.94	1.92	0.0166	-	0.520
d 42-49	2.22	2.29	2.33	2.27	2.27	2.40	2.16	2.27	0.0383	-	0.296
d 0-49	1.81	1.82	1.80	1.81	1.83	1.82	1.80	1.80	0.0105	-	0.241
Feed Intake (kg)											
d 0-14	7.14	7.16	7.17	7.06	7.21	7.15	7.16	6.96	0.076	-	0.354
d 14-21	7.895	7.804	7.985	7.849	8.122	7.895	8.212	7.804	0.124	-	0.131
d 14-28	17.06	17.01	17.88	17.06	18.01	17.29	17.79	17.38	0.281	-	0.060
d 28-42	28.72	29.08	28.77	28.61	30.17	29.04	29.27	28.06	0.495	-	0.170
d 42-49	15.79	16.38	15.61	15.79	16.56	15.79	16.38	15.70	0.284	-	0.105
d 0-49	68.74	69.65	69.46	68.56	71.96	69.28	70.60	68.10	1.013	-	0.174

Table 3.7 (Continued)

Mortality %											
d 0-14	0.00	1.19	1.79	1.19	0.00	0.60	0.60	0.00	0.691	-	0.479
d 14-21	1.78[a]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.595[ab]	0.00[b]	1.78[a]	0.528	1.487	0.032
d 14-28	2.97	0.00	1.19	0.00	0.595	0.595	0.595	2.38	0.789	-	0.079
d 28-42	0.00	0.00	1.78	1.78	0.00	1.19	0.00	1.19	0.843	-	0.465
d 42-49	0.00	0.00	0.595	0.00	0.00	0.00	0.595	0.00	0.300	-	0.552
d 0-49	2.97	1.19	4.16	2.38	0.59	2.38	1.78	2.97	1.233	-	0.567

[a-d]Means within the same row with no common superscript are significantly different

[1]Trt 1 Negative Control (No Additives), Trt 2 Positive Control (Live Coccidiosis Vaccine+No Additives), Trt 3 VM+SAL (Live Coccidiosis Vaccine+Virginiamycin+(Salinomycin d 14-28)), Trt 4 VM (Live Coccidiosis Vaccine+Virginiamycin), Trt 5 PRO+BMD (Live Coccidiosis Vaccine+Probiotic+Bacitracin Methylene Disalicylate), Trt 6 EB (Live Coccidiosis Vaccine+Essential Oil Blend), Trt 7 BMD (Live Coccidiosis Vaccine+BMD (Increased dose d 14-28)), Trt 8 BMD+SAL (LCV+Bacitracin Methylene Disalicylate+(Salinomycin d 14-28))

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at $P \leq 0.05$.

[5]Body weight gain.

References

1. Williams, R. B. 1999. A compartmentalized model for the estimation of the cost of Coccidiosis to the world's chicken production industry. Int. J. Parasitol. 29:1209-1229.

2. Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry Coccidiosis: Recent advancements in control measures and vaccine development. Expert Rev. Vaccines 5:143-163.

3. Shirley, M. W., A. L. Smith, and D. P. Baker. 2007. Challenges in the successful control of the avian coccidian. Vaccine. 25:5540-5547.

4. Chapman, H. D., T. K. Jeffers, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788-1801.

5. Williams, R. B. 2002. Fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Diseases. 46:775-802.

6. Mathis, G., J. Schaeffer, K. Cookson, J. Dickson, M. LaVorgna, and D. Waldrip. 2014. Effect of lasalocid or salinomycin administration on performance and immunity following coccidia vaccination of commercial broilers. J. Appl. Poult. Res. 23:577-585.

7. Ritzi, M. M., W. Abdelrahman, M. Mohnl, and R. A. Dalloul. 2014. Effects of probiotics and application methods on performance and response of broiler chickens to an Eimeria challenge. Poult. Sci. 93:2772-2778.

8. Bozkurt, M., N. Aysul, K. Küçükyilmaz ,, S. Aypak, G. Ege, A. U. Çatli, H. Aksit, F. Çöven, K. Seyrek, and M. Çınar. 2014. Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. Poult. Sci. 93:389-399.

9. Abdelrahman, M. Mohnl, K. Teichmann, B. Doupovec, G. Schatzmayr, B. Lumpkins, and G. Mathis. 2014. Comparative evaluation of probiotic and salinomycin effects on performance and coccidiosis control in broiler chickens. Poult. Sci. 93:3002-3008.

10. Chapman, H. D., and T. K Jeffers. 2014. Vaccination of chickens against coccidiosis ameliorates drug resistance in commercial poultry production. In. J. Parasitol. Drugs Design. 4:214-217.

11. Kinashi, H., N. Otake, H. Yonchara, S. Sato, and Y. Saito. 1973. The structure of salionmycin, a new member of the polyether antibiotics. Tetrahedron Letters. 49:4955-4958.

12. De Gussem, M. 2007. Coccidiosis in poultry: a review on diagnosis, control, prevention and interaction with overall gut health. 16th European Symposium on Poultry Nutrition.

13. Chapman, H. D. 1998. Evaluation of the efficacy of anticoccidial drugs against Eimeria species in the fowl. Int. J. Parasitol. 28:1141-1144.

14. Lee, S. H., H. S. Lillehoj, R. A. Dalloul, D. W. Park, Y. H. Hong, and J. J. Lin. 2007. Influence of *Pediococcus*-based probiotic on coccidiosis of broiler chickens. Poult. Sci. 86:63-66.

15. Abbas, R. Z., D. D. Colwell, and J. Gilleard. 2012. Botanicals: An alternative approach for the control of avian coccidiosis. World's Poult. Sci. J. 68:203-215.

16. Christaki, E., P. Florou-Paneri, I. Giannenas, M. Papazahariadou, N. A. Botsoglou, and A. B. Spais. 2004. Effect of mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. Anim. Res. 53:137-144.

17. Yang, Y., P. A. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J. 65:97-114.

18. Reisinger, N., T. Steiner, S. Nitsch, G. Schatzmayr, and T. J. Applegate. 2011. Effect of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. J. Appl. Poult. Res. 20:272-283.

19. Auburn University Poultry and Nutrition Center. Auburn, AL

20. Hatchpak Cocci III. Merial Inc. Gainesville, GA.

21. Phibro Animal Health Corporation, Teaneck, NJ

22. Zoetis. Florham Park, NJ.

23. Novus International, Inc. Saint Charles, MO.

24. Biomin Holding GmbH. Getzersdorf, Austria.

25. Aviagen. Huntsville, AL.

26. Peco Hatchery. Gordo, AL.

27. Protocol Formalin 10%. Fischer Scientific. Pittsburgh, PA.

28. Mississippi State University College of Veterinary Science. Mississippi State, MS.

29. Motic. Causeway Bay, Hong Kong.

30. Swift Optical Instruments, Inc. M10 Series. San Antonio, Texas.

31. Swift Optical Instruments, Inc. Digital camera attachment. San Antonio, Texas.

32. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.

33. Wang, X., E. D. Peebles, T. W. Morgan, R. L. Harkess, and W. Zhai. 2015. Protein source and nutrient density in the diets of male broilers from 8 to 21 d of age: Effect on small intestine morphology. Poult. Sci. 94:61-67.

34. Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and flood pen experiments with chickens. Exp. Parasitol. 28:30-36.

35. Brookfield Engineering Laboratories Inc. Model: LVDV-II+P CP. Middleboro, Massachusetts.

36. Corey, A. M., K. G. S. Wamsley, T. S. Winowiski, and J. S. Moritz. 2014. Effects of calcium lignosulfonate, mixer-added fat, and feed form on feed manufacture and broiler performance. J. Appl. Poult. Res. 23:418-428.

37. SAS version 9.2. SAS Institute Inc. Cary, NC.

38. Shires, A., J. R. Thompson, B. V. Turner, P. M. Kennedy, and Y. K. Goh. 1987. Rate of passage of corn-canola meal and corn-soybean meal diets through the gastrointestinal tract of broiler and White Leghorn chickens. Poult. Sci. 66:289-298.

39. Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476-485.

40. Lei, X. J., Y. J. Ru, and H. F. Zhang. 2014. Effect of *Bacillus amyloliquefaciens*-based direct-fed microbials and antibiotic on performance, nutrient digestibility, cecal microflora, and intestinal morphology in broiler chickens. J. Appl. Poult. Res. 23:486-493.

41. Tewari, A. K., and Maharana. 2011. Control of poultry coccidiosis: changing trends. J. Parasit. Dis. 35:10-17.

42. Miles, R. D., D. M. Janky, and R. H Harms. 1984. Virginiamycin and broiler performance. Poult. Sci. 63:1218-1221.

43. March, B. E., R. Soong, and C. MacMillan. 1978 Growth rate, feed conversion, and dietary metabolizable energy in response to virginiamycin supplementation of different diets. Poult. Sci. 57:1346-1350.

44. Jones, F. T. and S. C. Ricke. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. Poult. Sci. 82:613-617.

CHAPTER IV

EFFECTS OF VARYING COCCIDIOSIS CONTROL AND INCLUSION OF ALGAL BETA-1,3-GLUCAN ON DAY 0-59 MALE BROILER PERFORMANCE

Summary

Live coccidiosis vaccines (**LCV**) are commonly utilized in coccidiosis control programs. Drawbacks from their use can include the onset of necrotic enteritis or late cycling of oocysts. Beta-1,3-glucans, known for their immunomodulatory properties, may provide a means of reducing negative effects of LCV application as well as improve live performance of broilers. Therefore, the objective of the current study was to determine the effects of two new algal derived beta-1,3-glucan products (**ABG1** and **ABG2**) when utilized with or without existing coccidiosis control regimens, including a live coccidiosis vaccine (**LCV**), virginiamycin (**VM**), and/or the ionophore lasalocid (**LAS**) on live broiler performance, processing characteristics, and Immunoglobulin G (**IgG**) levels in blood serum. Birds that were fed Treatment (**Trt**) 1-6 were sprayed with a LCV at the hatchery, and fed diets utilizing no additives (**NA**), VM, VM+ABG1, ABG1 (50 g/t), ABG1 (100 g/t), and AGB2 respectively. Birds receiving Trt 7 and 8 were not sprayed with LCV and fed diets containing LAS+VM and LAS+VM+ABG1, respectively. Birds that were fed Trt 1 (NA) and 3 (VM+ABG1) demonstrated increased d 59 IgG blood serum levels. Live performance results indicate that birds receiving Trt 8 demonstrated consistent early live performance benefits of improved d 14 and 28 BW, d

14-28 BWG, d 0-14 FCR. Treatment 5 (ABG1) demonstrated increased total breast yield relative to d 59 body weight.

Keywords: beta glucan, antibiotics, ionophore regimens, broiler, processing

Description of Problem

Antibiotics and ionophore regimens have been used by the poultry industry to improve growth and performance by providing protection from pathogenic bacteria and parasitic infections [1-3]. Due to consumer push as well as increased pathogenic resistance to the previously mentioned controls, integrator's need for alternative products to improve broiler performance and negate adverse effects of LCV is of utmost concern [1]. The consumer's effect on the poultry market can be noted by the outlawing of antibiotics and ionophores as growth promoters in the European Union [4]. Consequently, the United States began following this trend in 2012 when the Food and Drug Administration established Guidance #209 and #213 stating that companies must remove growth promotion labels of commonly used antibiotics and change the existing label to prescription or Veterinary Feed Directive (**VFD**) status [5]. These two legislations will go into effect in December 2016; therefore it is imperative to research alternative products to help promote growth as well as increase immune responses to infectious pathogens.

There are many products that could serve as alternatives to existing medications such as probiotics, prebiotics, and herb and essential oil blends; however, in this paper beta-1,3-glucans are of interest. Beta-1,3-glucans are long chain polysaccharides typically found in the cell walls of yeast and fungi, though they are also extracted from bacteria, cereal grains, and algae. Each organism produces a polymer with a unique

structure and subsequently each has a unique immunological effect [6]. Algal beta-1,3-glucans (**ABG**) derived from *Euglena gracilis* are linear polymers of glucose subunits comprised almost exclusively of beta-1,3 linkages and contain no beta-1,6 linkages. They also accumulate within the cytoplasm of the cell as a pure crystalline granule. This distinguishes them from the branched structure of beta-1,3/1,6-glucans found in the cell wall of the yeast *Saccharomyces cerevisae* that are more commonly used in animal feeds [6]. Due to its linear form and the lack of beta-1,6 linkages, ABGs are thought to be more readily bioavailable and capable of demonstrating immunmodulatory and growth performance benefits [7]. While effects of ABG are relatively unknown in poultry research, many studies have demonstrated the effects of the aforementioned yeast derived beta glucans on the innate and adaptive immune responses [8-12]. Benefits include enhancing the proliferation and phagocytic capabilities of macrophages as well as magnifying the levels of immunoglobulin G (**IgG**) found in blood serum [9, 10, 12]. Additionally, in comparable monogastric research (swine), improvements have been reported in regards to BW and feed efficiency [13, 14].

Despite the well-documented use of yeast beta glucans in poultry research, little is known about ABG and their effects on live performance when utilized alone or with existing coccidiosis controls. Therefore, the premise of this study was to determine the effects of a new ABG when used with and without existing coccidiosis controls on live performance, processing characteristics, and Immunoglobulin G levels in blood serum.

Materials and Methods

Diet Preparations

Diets utilized in the current study were of proprietary commercial formulation and initially batched without feed additives at Mississippi State University Poultry Research Unit. Basal diets were corn-soybean meal based, containing meat and bone meal. Analyzed diet composition can be found in Table 4.1. Starter phase diets were fed in the form of crumbled pellets from d 0 to 14. Grower, finisher 1, finisher 2, and withdrawal feeds were fed in the form of intact pellets from d 14-28, 28-46, 46-52, and 52-59, respectively. Prior to pelleting, feed additives were added into each respective treatment in a smaller mixer containing approximately 11 kg of the basal diet. This small batch was then mixed for five minutes and then added to the large mixer containing the remaining basal diet for each respective treatment to be pelleted.

Dietary treatments and feed additive inclusions are displayed in Table 4.2. Briefly, feed additives utilized in this study included virginiamycin 15g/t (**VM**) [15], lasalocid 68g/t (**LAS**) [16], an algal derived beta glucan zinc metal polysaccharide 100g/t (**ABG1**) [17], and a second algal derived beta glucan polysaccharide (**ABG2**) 90g/t [17]. As a reminder, birds fed Trt 1-6 were sprayed with a LCV [18] at the hatchery, while birds fed Trt 7 and 8 we left unsprayed to compare the use of LCV with inclusions of VM, ABG1 and ABG2 vs. an ionophore regimen and ABG1. All feed additives were included at the manufacturer's recommendation except Trt 4 utilized an inclusion of 50g/t ABG1.

Bird Husbandry

All animal use was approved by the Mississippi State University Institutional Animal Care and Use Committee. One thousand five hundred and thirty six day-old chicks (Ross × Ross 708) [19] male broilers were used for this experiment. The experimental house was a solid walled broiler research house at Mississippi State University Poultry Research Unit. Chicks were obtained from a commercial hatchery [20] and vaccinated for Mareck's disease and Newcastle disease at the hatchery. For treatments requiring LCV, application was accomplish at the hatchery via spray. Upon arrival from the hatchery, chicks were feather-sexed so that 16 males could be randomly allocated to one of 96 floor pens. At d 0, pen size was decreased using a mesh divider ($0.052 m^2$/bird). On d 14, pen dividers were removed to return the pen area to the original size ($0.70 m^2$/bird). Each floor pen was equipped with a hanging commercial feeder, a nipple drinker line (3 nipple drinkers/pen), and top dressed with used litter obtained from a commercial poultry house at Mississippi State University. Feed and water were offered ad libitum throughout the trial.

On d 0 temperature for the research house started at 32.22°C and decreased incrementally until a final temperature of 16.11°C was reached on d 49 and maintained until d 59. Birds received full light intensity, 32.3 lux, from d 0 to 7 with 24L:0D. On d 10 light intensity was decreased incrementally until a light intensity of 21.5 lux was reached at d 25 with 16L:8D. On d 26 light intensity was again decreased incrementally until a light intensity of 5.4 lux was reached at d 32 with 18L:6D. Finally, on d 33 light intensity was incrementally decreased until a final light intensity of 3.4 lux was reached on d 40 and maintained until d 59 with 20L:4D.

Blood Serum Analysis for IgG

On d 28 and 59, two birds were randomly chosen and removed from each pen. Blood was collected using a sterile 22 gauge needle and a 5 ml syringe from the brachial vein, stored in a red top vial [21], and then allowed to clot for 2 hours. The tubes were then subsequently centrifuged at 5000 RPM for 5 minutes. Blood serum was then pooled by pen for each respective pen and stored in a -80˚ C until IgG levels could be determined.

Blood serum was then thawed in a refrigerator the day prior to IgG determination. For the determination we utilized a Chicken IgG ELISA Kit [22] and followed the manufacturer's protocol. Select treatments were analyzed due to benefits observed during the early phases of growth; these treatments included Trt 1 (LCV+NA), 3 (LCV+VM+ABG1), 5 (LCV+ABG1), 7 (LAS+VM), and 8 (LAS+VM+ ABG1).

Measured Live Performance Variables

Live performance variables included the following: average (**AVG**) BW, body weight gain (**BWG**), pen feed intake (**FI**), and FCR for d 0-14, 14-28, 28-46, 46-52, and 52-59, as well as d 0-59. Mortality was collected daily and saved for necropsy. Mortality weights were used to calculate adjusted FCR.

Processing Characteristics

On d 59, four birds per pen were tagged, weighed, and cooped. The following day, d 60, birds were taken to the Mississippi State University Poultry Research Unit Processing Center. At processing, carcass weights and abdominal fat pad weights were recorded. Boneless and skinless breast, tender, drum, thigh, and wings were obtained by

manually deboning the carcass after 4 hours of chilling in an ice bath. Processing characteristics were reported as an average yield relative to the d 59 body weight.

Statistical Analysis

A randomized complete block design was used with 8 treatments (12 replicates of each) being equally allocated to each block. Treatments were blocked by location. The experimental unit was considered to be one floor pen of birds. The statistical analysis utilized was the GLM procedure in SAS [23]. Multiple comparisons were made between all treatment means using Fisher's least significant difference test after on-way ANOVA was determined. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Effects on Immunoglobulin G Concentrations (IgG)

Day 28 IgG blood serum levels were not significantly affected by any of the selected dietary treatments ($P > 0.05$; Table 4.3). However, on d 59, birds that were fed Trt 1 (LCV+NA) and 3 (LCV+VM+ABG1) demonstrated increased levels of IgG found in blood serum ($P > 0.0001$; Table 4.3) when compared to the remaining selected Trt (5 (LCV+ABG1), 7 (LAS+VM), and 8 (LAS+VM+ ABG1). In comparison to current results, yeast derived beta glucan supplementation has demonstrated magnification of IgG in blood serum on d 21 and 42 in chickens [9]. A mammalian study conducted on splenocytes from mice also demonstrated increased IgG production when administered a beta-glucan [24]. Different degrees of branching, linkages, solubility, and overall structure of beta glucans can affect the immune modulation characteristics [6]. Therefore, the source of the beta-glucan product could provide explanation of the

differences observed in the current research and previous literature. Additionally, Cox and cohorts [11] suggests that immunomodulation functions of beta-glucans may not be significant unless under a significant challenge, which could be an explanation for the current study. Additionally, the ionophore regimen (LAS+VM) in Trt 7 did not demonstrate significant increases in IgG levels at d 59, further suggesting that a significant challenge was not present.

Bird Performance

During the discussion of results comparisons of significant differences will be made between treatments that consistently improved live performance due to interests of integrators and determining the most effective inclusion strategies during a broiler grow-out. Mortality % results will not be discussed due to the non-significance ($P>0.05$) during each growth phase as well as the cumulative grow-out. Pen FI was only significantly affected by Trt on d 28-46 ($P=0.0008$) and will only be discussed when describing the BWG trend observed during d 28-46 ($P=0.0554$). However, these data are reported in Table 4.4.

BW Effects

Treatment 8 (LAS+VM+ABG1) demonstrated significant effects on Avg. BW/bird on d 14 and 28 ($P=0.0227$ and $P=0.0001$, respectively; Table 4.4). On each of these days birds fed Trt 8 (LAS+VM+ ABG1) demonstrated the most improved BW when compared to the remaining seven treatments. We must note, birds fed Trt 1 (LCV+NA), 2 (LCV+VM), 6 (LCV+ABG2), and 7 (LAS+VM) yielded statistically similar d 14 BW to Trt 3 (LAS+VM+ABG1) but this performance was not consistent at

the next weigh day, d 28. On d 28, birds fed Trt 8 (LAS+VM+ABG1) again demonstrated the largest improvement to BW (P=0.0001; Table 4.4). However, this time birds fed Trt 7 (LAS+VM) was the only similar Trt in regards to d 28 BW. Comparing effects of Trt 8 to Trt 7 (LAS+VM) on d 14 and 28 BW improvements, we can note the addition of ABG1, yielded a numerically higher BW when compared to birds fed Trt 7 (Table 4.4). Furthermore, comparing the improvements of Trt 8 (LAS+VM+ABG1) vs. Trt 3 (LCV+VM+ABG1) on d 14 and 28 BW can also indicate the efficacy of utilizing LAS instead of the LCV (P<0.05; Table 4.4).

Body weight gain was only significantly improved during d 14-28 (P=0.0006; Table 4.4). Birds that were fed Trt 8 (LAS+VM+ABG1) demonstrated the largest BWG when compared to the remaining seven treatments. It should be noted again that Trt 7 (LAS+VM) demonstrated statistically similar results to Trt 8 (LAS+VM+ABG1), however we can still note the improvements provided by inclusion of ABG1 by the numerically higher BWG for Trt 8 (LAS+VM+ABG1). Though not significant, a strong trend was reported for d 28-46 BWG (P=0.0554). Despite the early growth performance improvements (BW and BWG) exhibited by Trt 8 (LAS+VM+ABG1), birds fed this Trt had the lowest BWG during d 28-46. In all likelihood, it is correlated to the significantly decreased Pen FI of Trt 8 (LAS+VM+ABG1) during this phase (P=0.0008; Table 4.4).

To our knowledge the effects of an ABG when used with or without existing coccidiosis controls has not been reported. However, these feed additives and controls have been individually researched under many conditions. The use of live coccidiosis vaccines has been incorporated into management procedures for many years due to the ability to stimulate immune responses, thus reducing primary infections of *Eimeria*

parasites [25, 26]. Virginiamycin is another commonly used control and has been proven to improve BW in chickens [27, 28]. The effects of LAS inclusion have been duly noted in several studies reporting increased BW in broilers when basal diets incorporated LAS [29-31]. Finally, ABG research is scarce due to the recent advancements in production; however, research has been conducted on beta-glucans derived from yeast. Recent studies have found conflicting results. Zhang et al. [9] found that inclusion of *S. cerevisiae* beta-glucan significantly improved BW when compared to diets lacking the beta-glucan during the first 3 weeks of growth. However, contrary to the current study and results reported by Zhang and cohorts, research has demonstrated that beta-glucans may not have any effects, positive or negative, on BW or BWG [11, 32].

FCR Effects

During d 0-14 birds fed Trt 8 (LAS+VM+ABG1) had the most improved FCR when compared to the remaining treatments (P=0.0217; Table 4.4). Again, Trt 7 (LAS+VM) FCR can be noted as being statistically similar yet numerically higher. Additionally, Trt 1 (LCV+NA) and 3 (LCV+VM+ABG1) demonstrated statistically similar FCR results to Trt 8 (LAS+VM+ABG1); however, the use of LAS vs. LCV again demonstrated improved live performance as represented as a numerically lower FCR. Birds fed Trt 7 (LAS+VM) during d 14-28 demonstrated significant improvements for FCR (P=0.0017; Table 4.4). The bird's FCR was significantly lower than all remaining treatments aside from Trt 8, which was statistically similar but numerically higher. In accordance with the current study, beta-glucans derived from yeast have found to improve FCR during d 0-21 and 21-42 [8]. We speculate that the immunomodulator effects of beta-glucans, as reported in previous literature [8-12], could allow for an

energy sparing effect, thus allowing feed to be efficiently utilized for growth. Additionally, a second study conducted by Cox and cohorts [33] demonstrated yeast derived beta-glucans effectively reducing lesion severity in the intestine of *Eimeria* infected chicks. While this did not directly affect the live performance in their study, the decreased intestinal damage could possibly allow for better nutrient absorption demonstrated by improved FCR from supplementation of ABG in this current study.

Processing Characteristics

Day 60 total breast yield relative to live BW was the largest in birds that were fed Trt 5 (LCV+ABG1) when compared to the remaining treatments (P=0.042; Table 4.5). These results are similar to breast yield data reported that demonstrate increased breast yield at d 35 in birds fed diets containing a beta-1,3/1,6-glucan [34]. It is unclear how ABG supplementation can affect processing yields; however we can speculate that immunomodulator effects of ABG1 could allow for the partitioning of nutrients to be more directed to the growth instead of the young immune system. The remaining processing variables were not affected by the dietary treatments but are displayed in Table 4.5.

Overview

During the first few weeks of chick growth the avian immune system is not fully matured and is still relying on yolk sac antibodies. As previously mentioned, the immunomodulating characteristics described by Volman and colleagues [6] could allow for energy to be utilized for tissue growth. Furthermore, the addition of LAS and VM may help improve performance by combating infectious pathogens and maintaining a

normal gut microflora. Current results suggest that integrators interested in using an alternative strategy of coccidiosis control could supply beta-glucans derived from algae into their poultry rations. This had the greatest positive effects on d 0-14 and 14-28 live performance when used in combination with LAS and VM. However, the inconclusiveness of data regarding beta-glucan inclusion in the current study and previous research suggests more research is warranted in the area of utilizing beta-glucans in combination with select coccidiosis controls during a phase feeding grow-out.

Conclusions and Applications

1. Immunoglobulin G concentrations found in blood serum were magnified with the addition of ABG1 to LCV+VM (Trt 3) on d 59; it is possible that future studies utilizing a more pronounced challenge would exhibit further treatment separation.
2. Birds in the current study fed diets containing LAS+VM+ABG1 (Trt 8) demonstrated significant improvements in d 14 and 28 BW, d 14-28 BWG, and d 0-14 and 14-28 FCR. However, these did not translate to cumulative performance differences. We suggest that future research evaluate a feeding regime wherein algal beta glucan are only fed in the starter and grower feed, then withdrawn.
3. Birds fed diets containing LAS+VM (Trt 7) demonstrated similar results to those fed LAS+VM+ABG1 (Trt 8) in regards to d 14 and 28 BW, d 14-28 BWG, and d 0-14 and 14-28 FCR.

4. Yield of total breast relative to live BW was significantly increased with the dietary inclusion of ABG1 (Trt 5) when compared to the remaining Trts.

Table 4.1 Analysis of Basal Diets

Nutrient[1]	Phase of Growth				
	Starter (d0-14)	Grower (d14-28)	Finisher 1 (d28-46)	Finisher 2 (d46-52)	Withdrawal (d 52-59)
Lysine	1.30	1.24	1.23	1.05	0.94
Methionine	0.61	0.60	0.52	0.54	0.50
Cysteine	0.38	0.37	0.30	0.27	0.27
Threonine	0.82	0.79	0.72	0.61	0.59
Isoleucine	0.90	0.87	0.77	0.63	0.63
Leucine	1.77	1.72	1.61	1.39	1.37
Proline	1.33	1.24	1.11	0.99	0.98
Glycine	1.21	1.11	1.00	0.86	0.83
Alanine	1.13	1.08	1.04	0.93	0.89
Valine	1.08	1.06	0.93	0.79	0.79
Aspartic Acid	2.16	2.08	1.86	1.49	1.50
Glutamic Acid	3.57	3.45	3.21	2.67	2.66
Crude Protein %[2]	22.669	21.071	20.065	17.247	17.321
Gross Energy (kcal/kg)[3]	4022.79	3994.97	3991.84	3998.17	3975.77

[1]CML+9. JAOAC 70:171-174, 1987. Agricultural Experiment Station University of Missouri-Columbia.
[2]Determined by LECO 528 Nitrogen Determinator at Mississippi State University
[3]Determined by Parr Bomb Calorimeter at Mississippi State University.

Table 4.2 Dietary Treatment Outline

Treatment	LCV[1] (+ or -)[2]	Starter (d 0-14)	Grower (d 14-28)	Finisher 1 (d 28-46)	Finisher 2 (d 46-52)	Withdrawal[3] (d 52-59)
1	+	NA	NA	NA	NA	NA
2	+	VM[4]	VM	VM	VM	NA
3	+	VM+ABG1[5]	VM+ABG1	VM+ABG1	VM+ABG1	NA
4	+	ABG1 (50g/t)[6]	ABG1 (50g/t)	ABG1 (50g/t)	ABG1 (50g/t)	NA
5	+	ABG1	ABG1	ABG1	ABG1	NA
6	+	ABG2[6] (90g/t)	ABG2 (90g/t)	ABG2 (90g/t)	ABG2 (90g/t)	NA
7	-	LAS[8]+VM	LAS+VM	LAS+VM	VM	NA
8	-	LAS+VM+ABG1	LAS+VM+ABG1	LAS+VM+ABG1	VM+ABG1	NA

[1]Live coccidosis vaccine. [18]
[2]+ represents chicks were sprayed with LCV. – represents chicks were not sprayed with LCV.
[3]Withdrawal diets did not have feed additive inclusions.
[4]Virginiamycin (15g/t) utilized throughout study until d 52. [15]
[5]Algal derived beta-1,3-glucan product 1. 100g/t throughout study unless noted otherwise. [17]
[6]Treatment 4 utilized 50g/t of ABG1 throughout the study. [17]
[7]Algal derived beta-1,3-glucan product 2. 90g/t throughout study unless noted otherwise. [17]
[8]Lasalocid sodium ionophore. 68g/t throughout study until d 46. [16]

Table 4.3 Effects of Dietary Treatments on Blood Serum Immunoglobulin G Concentrations

Variable	Treatment[1]					SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	3	5	7	8			
d 28 IgG[5] (ng/ml)	33.90	37.62	55.88	30.00	44.62	9.444	-	0.3697
d 59 IgG (ng/ml)	24.078[a]	23.169[a]	13.688[b]	14.899[b]	13.877[b]	1.708	4.8693	<0.0001

[a-d]Means within the same row with no common superscript are significantly different

[1]Trt 1 (Live Coccidiosis Vaccine+No Additives), Trt 3 (Live Coccidiosis Vaccine +Virginiamycin+ Algal Beta-Glucan 1), Trt 5 (Live Coccidiosis Vaccine + Algal Beta-Glucan 1), Trt 7 (Lasalocid+Virginiamycin), Trt 8 (Lasalocid+Virginiamycin+Algal Beta-Glucan 1)

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at $P \leq 0.05$.

[5]Immunoglobulin G.

Table 4.4 Effects of dietary treatments on broiler live performance

Variable	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Avg. BW (kg)											
d 14	0.371^{ab}	0.377^{ab}	0.367^{bc}	0.364^{bc}	0.353^{c}	0.369^{abc}	0.373^{ab}	0.385^{a}	0.0017	0.0051	0.0227
d 28	1.277^{c}	1.302^{bc}	1.284^{c}	1.289^{c}	1.275^{c}	1.279^{c}	1.338^{ab}	1.355^{a}	0.0137	0.0411	0.0001
d 46	2.840	2.926	2.831	2.785	2.849	2.808	2.813	2.799	0.0113	-	0.379
d 52	3.448	3.638	3.620	3.539	3.598	3.548	3.543	3.570	0.0154	-	0.932
d 59	4.074	4.019	4.006	3.983	4.047	3.970	4.024	4.092	0.0285	-	0.582
Avg. BW Gain (kg)											
d 0-14	0.3348	0.3371	0.3294	0.3325	0.3198	0.3307	0.3352	0.3461	0.0015	-	0.1270
d 14-28	0.907^{c}	0.913^{c}	0.917^{c}	0.934^{bc}	0.923^{c}	0.919^{c}	0.958^{ab}	0.974^{a}	0.0034	0.0102	0.0006
d 28-46	1.568	1.580	1.561	1.513	1.565	1.593	1.493	1.420	0.0055	-	0.0554
d 46-52	0.648	0.639	0.666	0.662	0.662	0.671	0.635	0.617	0.0082	-	0.0817
d 52-59	0.476	0.449	0.476	0.444	0.476	0.444	0.494	0.512	0.0286	-	0.582
d 0-59	3.934	3.897	3.902	3.875	3.952	3.866	3.825	3.857	0.0204	-	0.971
FCR											
d 0-14	1.328^{ab}	1.353^{a}	1.329^{ab}	1.366^{a}	1.367^{a}	1.355^{a}	1.333^{ab}	1.297^{b}	0.004	0.0428	0.0217
d 14-28	1.642^{ab}	1.678^{a}	1.659^{ab}	1.646^{ab}	1.660^{ab}	1.651^{ab}	1.581^{c}	1.618^{bc}	0.005	0.0458	0.0017
d 28-46	2.06	2.07	2.07	2.13	2.06	2.11	2.11	2.17	0.009	-	0.126
d 46-52	1.85	1.87	1.78	1.76	1.73	1.86	1.89	1.88	0.028	-	0.882
d 52-59	3.06	3.29	3.03	3.22	3.27	3.57	3.01	2.95	0.054	-	0.188
d 0-59	1.95	1.97	1.96	1.96	1.96	1.97	1.95	1.95	0.044	-	0.900
Pen Feed Intake (kg)											
d 0-14	7.037	7.186	6.969	7.014	6.878	7.114	7.064	7.291	0.0318	-	0.2226
d 14-28	23.443	24.373	23.824	23.548	24.228	23.793	23.901	24.392	0.1002	-	0.3524
d 28-46	44.895^{a}	44.668^{ab}	44.382^{ab}	43.715^{ab}	44.373^{ab}	45.707^{a}	42.853^{ab}	40.993^{c}	0.2070	0.621	0.0008
d 46-52	14.142	13.312	13.543	12.962	13.516	13.607	13.166	12.323	0.1198	-	0.152
d 52-59	16.079	15.689	15.739	15.113	15.966	15.952	15.943	15.204	0.1438	-	0.818
d 0-59	105.195	105.227	104.456	102.886	104.882	106.180	102.931	100.209	0.4532	-	0.217
Mortality %											
d 0-14	1.04	2.08	0.52	2.08	1.56	0.57	1.04	0.00	0.195	-	0.3093
d 14-28	1.56	2.08	1.04	4.17	2.08	1.14	2.08	3.13	0.293	-	0.4226
d 28-46	1.79	3.57	2.98	4.76	2.98	1.95	3.57	5.36	0.396	-	0.619
d 46-52	0.76	0.00	1.45	2.97	0.00	1.58	2.08	1.39	0.292	-	0.414
d 52-59	1.39	1.45	0.69	0.93	3.54	3.10	0.69	1.39	0.349	-	0.561
d 0-59	3.13	4.17	4.17	6.77	5.21	5.11	5.21	6.77	0.396	-	0.632

[a-d]Means within the same row with no common superscript are significantly different

[1]Trt 1 (Live Coccidiosis Vaccine+No Additives), Trt 2 (Live Coccidiosis Vaccine+Virginiamycin), Trt 3 (Live Coccidiosis Vaccine +Virginiamycin+Algal Beta-Glucan 1), Trt 4 (LCV+Algal Beta-Glucan 1 (50g/t)), Trt 5 (Live Coccidiosis Vaccine + Algal Beta-Glucan 1), Trt 6 (LCV+Algal Beta-Glucan 2), Trt 7 (Lasalocid+Virginiamycin), Trt 8 (Lasalocid+Virginiamycin+Algal Beta-Glucan 1)

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at P≤0.05.

Table 4.5 Effects of dietary treatments on d 60 processing yields relative to d59 live BW

Yield Relative to d 59 BW (%)	Treatment[1]								SEM[2]	Fisher's LSD[3]	P-Value[4]
	1	2	3	4	5	6	7	8			
Carcass	74.33	74.77	74.44	74.39	74.58	74.28	73.56	76.362	0.399	-	0.322
Total Breast[5]	24.73^{ab}	24.26^{abc}	23.78^{bc}	23.65^{bc}	25.17^{a}	24.06^{bc}	23.73^{bc}	23.57^{c}	0.385	1.08	0.042
Breast[6]	20.65	20.02	19.69	19.63	20.35	19.93	19.81	19.55	0.262	-	0.055
Tender[7]	4.08	4.25	4.09	4.01	4.82	4.13	3.92	4.01	0.264	-	0.349
Drumstick	9.56	9.56	9.74	9.85	9.62	9.60	9.38	9.40	0.142	-	0.294
Thigh	12.23	12.17	12.47	12.28	12.10	11.59	11.99	12.31	0.156	-	0.310
Wing	7.89	8.19	7383	7.99	7.92	8.03	7.65	7.81	0.162	-	0.441
Abdominal Fat Pad	1.22	1.24	1.25	1.27	1.14	1.21	1.23	1.32	0.083	-	0.884

[a-d]Means within the same row with no common superscript are significantly different

[1]Trt 1 (Live Coccidiosis Vaccine+No Additives), Trt 2 (Live Coccidiosis Vaccine+Virginiamycin), Trt 3 (Live Coccidiosis Vaccine +Virginiamycin+Algal Beta-Glucan 1), Trt 4 (LCV+Algal Beta-Glucan 1 (50g/t)), Trt 5 (Live Coccidiosis Vaccine + Algal Beta-Glucan 1), Trt 6 (LCV+Algal Beta-Glucan 2), Trt 7 (Lasalocid+Virginiamycin), Trt 8 (Lasalocid+Virginiamycin+Algal Beta-Glucan 1)

[2]Standard Error of the Mean.

[3]Fisher's Least Significant Difference.

[4]Significance level set at $P \leq 0.05$.

[5]Total Breast includes pectoralis major and minor.

[6]Breast refers to pectoralis major.

[7]Tender refers to pectoralis minor.

References

1. Donaghue, D. J. 2003. Antibiotics residues in poultry tissues and eggs: human health concerns? Poult. Sci. 82:618-612.

2. Chapman, H. D., T. K. Jeffers and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788-1801.

3. Jones, F. T. and S. C. Ricke. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. Poult. Sci. 82:613-617.

4. Castanon, J. I. R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466-2471.

5. The Poultry Site. US Poultry Vets Face Challenge with New Antibiotic Regulations. 6/2/15. http://www.thepoultrysite.com/poultrynews/33325/us-poultry-vets-face-challenge-with-new-antibiotic-regulations/

6. Volman, J. J., J. D. Ramakers, and J.Plat 2008. Dietary modulation of immune function by beta glucans. Physiol. Behav. 94:276-284

7. Levine, R. B. 2014. Algal immunomodulator may improve broiler health. Feedstuffs. June 24, 2014.

8. Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, T. W. Hahn. 2006. Effects of the supplementation of beta glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291-298.

9. Zhang, B., Y. Guo, and Z. Wang. 2008. The modulating effect of beta-1,3/1,6-glucan supplementation in the diet on performance and immunological responses of broiler chickens. Asian-Aust. J. Anim. Sci. 21:237-244.

10. Guo, Y., R. A. Ali, and M. A. Qureshi. 2003. The influence of beta glucan on immune responses in broiler chicks. Immunopharmacol. Immunotoxicol. 25:461-472.

11. Cox, C. M., L. H Stuard, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. 2010. Performance and immune responses to dietary beta glucan in broiler chicks. Poult. Sci. 89:1924-1933.

12. Chen, K. L., B. C. Weng, M. T. Chang, Y. H. Liao, T. T. Chen, and C. Chu. 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by beta-1,3-1,6-glucan. Poult. Sci. 87:2242-2249.

13. Hiss, S. and H Sauerwein.2003. Influence of dietary beta glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentrations in pigs. J. Anim. Physiol. a. Anim. Nutr. 87:2-11.

14. Li, J., D. F. Li, J. J. Xing, Z. B. Cheng and C. H. Lai. 2006. Effects of beta glucan extracted from Saccharomyces cerevisiae on growth performance, and immunological and somatotropic responses of pigs challenged with Escherichia coli lipopolysaccharide. J. Anim. Sci. 84:2374-2381.

15. Phibro Animal Health Corporation. Teaneck, NJ.

16. Zoetis. Florham Park, NJ.

17. Algal Scientific Corporation. Plymouth, MI.

18. Merial Inc. Gainesville, GA.

19. Aviagen. Huntsville, AL.

20. Peco Hatchery. Gordo, AL.

21. Serum blood collection tubes. Becton, Dickinson and Company. Franklin Lakes, NJ.

22. Chicken IgG ELISA Kit. Bethyl Laboratories, Inc.

23. SAS version 9.2. SAS Institure Inc. Cary, NC.

24. Suzuki, Y., Y. Adachi, N. Ohno, and T. Yadomae. 2001. Th1/Th2-balancing immunomodulating activity of gel-forming (1-3)-beta-glucans from fungi. Biol. Pharm. Bull. 24:811-819.

25. Shirley, M. W. A. L. Smith, and D. P. Baker. 2007. Challenges in the successful control of the avian coccidian. Vaccine. 25:5540-5547.

26. Trout, J. M., and H. S. Lillehoj. 1996. T lymphocyte roles during Eimeria acervulina and Eimeria tenella infections. Vet. Immunol. Immunopathol. 53:163-172.

27. Miles, R. D., D. M Janky, and R. H, Harms. 1984. Virginiamycin and broiler performance. Poult. Sci. 63:1218-1221.

28. Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quanitatitve morphology. Poult. Sci. 85:476-485.

29. Bains, B. S. 1980. Lasalocid efficacy in the prevention of coccidiosis of broiler chickens under floor pen conditions. Poult. Sci. 59:63-68.

30. Stanley, V. G., C. Gray, M. Daley, W. F. Krueger, and A. E. Sefton. An alternative to antibiotic-based drugs in feed for enhancing performance of borilers grown on Eimeria spp.-infected litter. Poult. Sci. 83:39-44.

31. Mathis, G., J. Schaeffer, K. Cookson, J. Dickson, M. LaVorgna, and D. Waldrip. 2014. Effect oflasalocid or salinomycin administration on performance and immunity following coccidian vaccination of commercial broilers. J. Appl. Poult. Res. 23:577-585.

32. Morales-Lopez, R., E. Auclair, F. Garcia, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; beta-1,3/1,6-glucans; and mannoproteins in broiler chicken diets. Poult. Sci. 88:601-607.

33. Cox, C. M., L. H. Sumners, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. Immune responses to dietary beta-glucan in broiler chicks during an Eimeria challenge. Poult. Sci. 89:2597-2607.

34. Cho, J. H., Z. F. Zhang, and L. H. Kim. 2013. Effects of single or combined dietary supplementation of beta-glucan and kefir on growth performance, blood characteristics, and meat quality in broilers. Br. Poult. Sci. 54:216-221.